Pure and simple?

Understanding pure shift NMR methodology

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September 18th, 2017

SMASH, Baveno, Italy

MANCHESTER 1824

The University of Manchester

SMASH

Small Molecule NMR Conference

Outline

I - Introduction:

Pure shift NMR: setting the scene

Acquisition methods

“Active spin refocusing” methods

Implementation

II - Applications

Structure analysis

Diffusion studies

Measurement of couplings

Mixture analysis

Enantiomeric studies

Dynamic processes

III - Practical aspects:

Sensitivity

Spectral quality

Others

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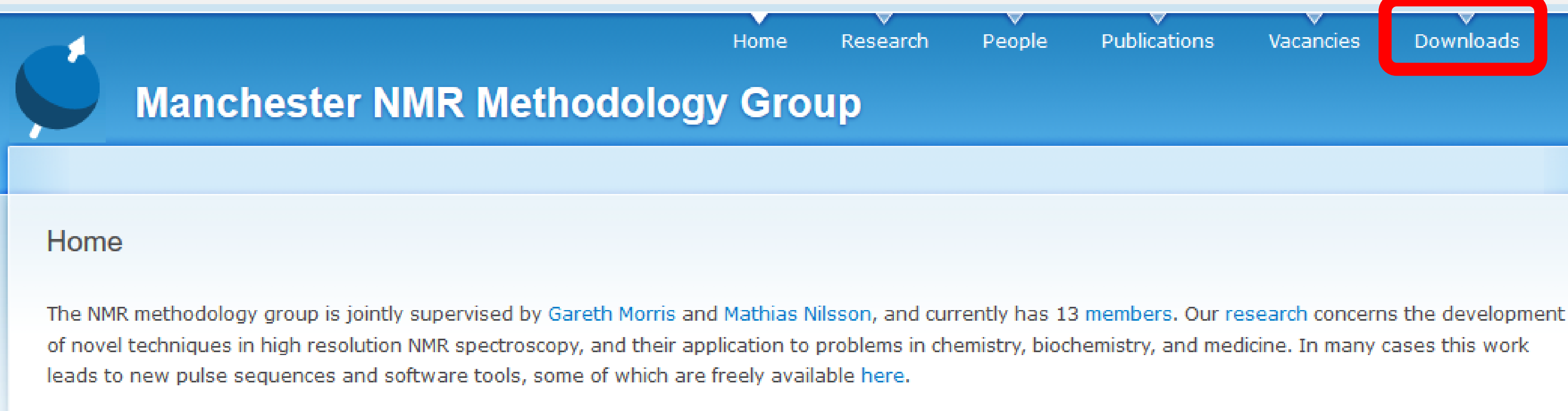

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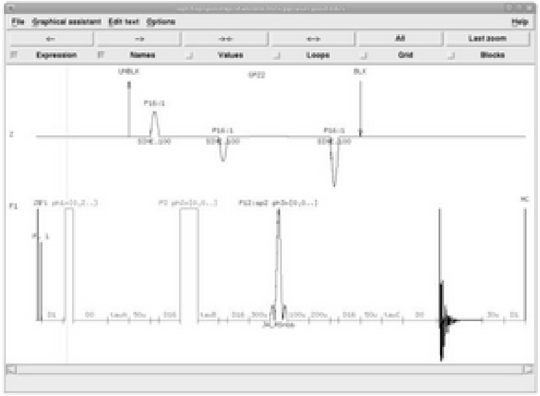
Home

Downloads

Pulse Sequences

We are currently preparing many of our pulse sequences, parameter sets, example datasets and processing macros for the website. Some are available here but if you would like to use any of the other the sequences, as described in the publications section, please email us. The majority of sequences are available for Varian systems and we are gradually writing the Bruker variants.

The pulse sequences and any macros required for data conversion can be accessed from this part of the website.



Workshops and presentations

The slides from some of the workshops and presentations given by group members are available from this part of the website. There is a pure shift NMR package available for download as part of our 2017 workshop on pure shift NMR.

Download from our website:

http://nmr.chemistry.manchester.ac.uk

Workshops and Presentations

Workshops

Pure shift NMR workshop - SMASH 2017

Manchester 2017 - Pure Shift NMR Workshop

SMASH 2014 - Pure Shift NMR Workshop

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What *is* pure shift NMR?

Conventional
^{1}H NMR

✓ Chemical shifts (δ)

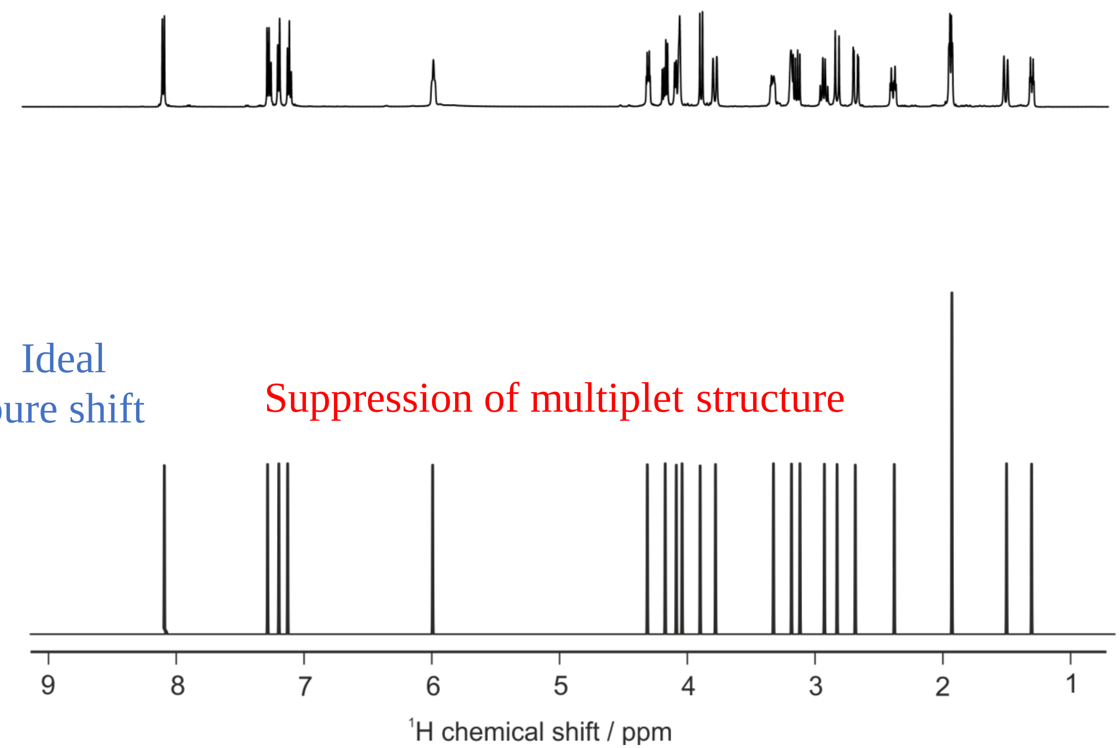
✓ Homonuclear couplings (J_{HH})

Ideal
pure shift

Suppression of multiplet structure

✓ Chemical shifts (δ)

✗ Homonuclear couplings (J_{HH})

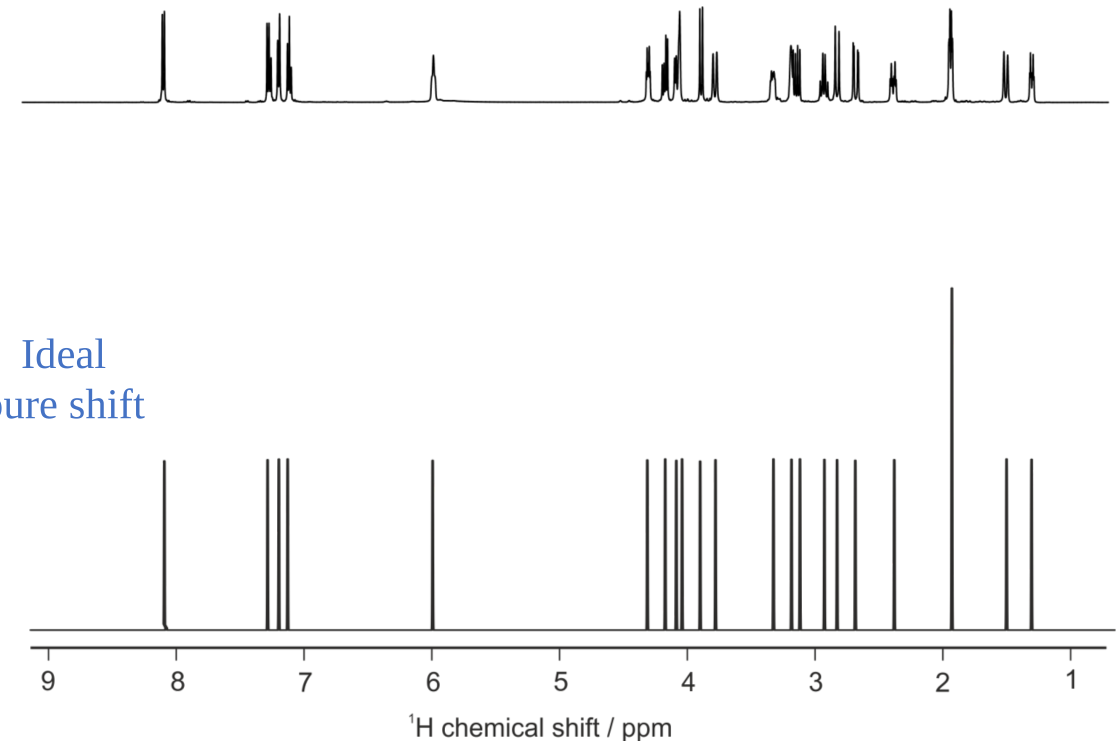


A pure shift spectrum is one in which peak positions are determined solely by chemical shifts

Why is pure shift NMR useful?

Conventional
^{1}H NMR

Ideal
pure shift

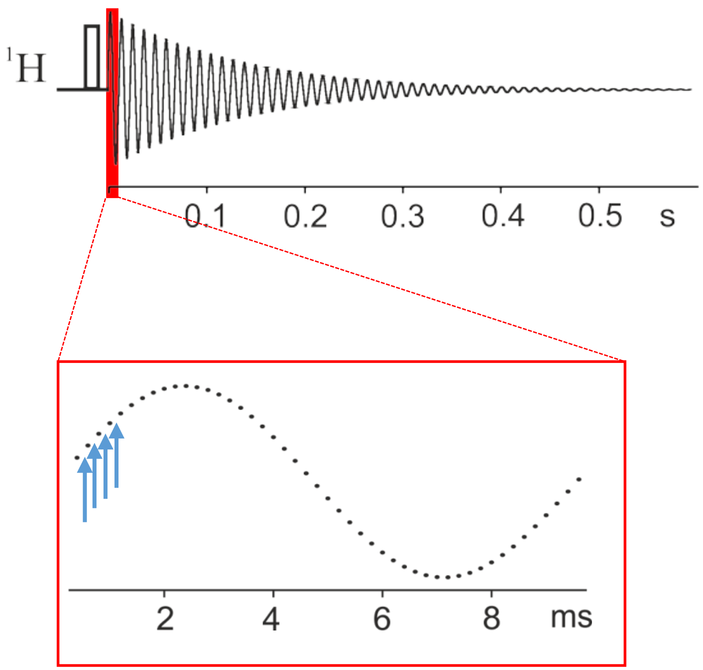


- ✗ Signal overlap
- ✗ Poor resolution
- ✗ Challenging spectral analysis

- ✓ Reduced spectral complexity
- ✓ Enhanced signal resolution
- ✓ Easier spectral analysis

How could we get a “perfect” pure shift spectrum?

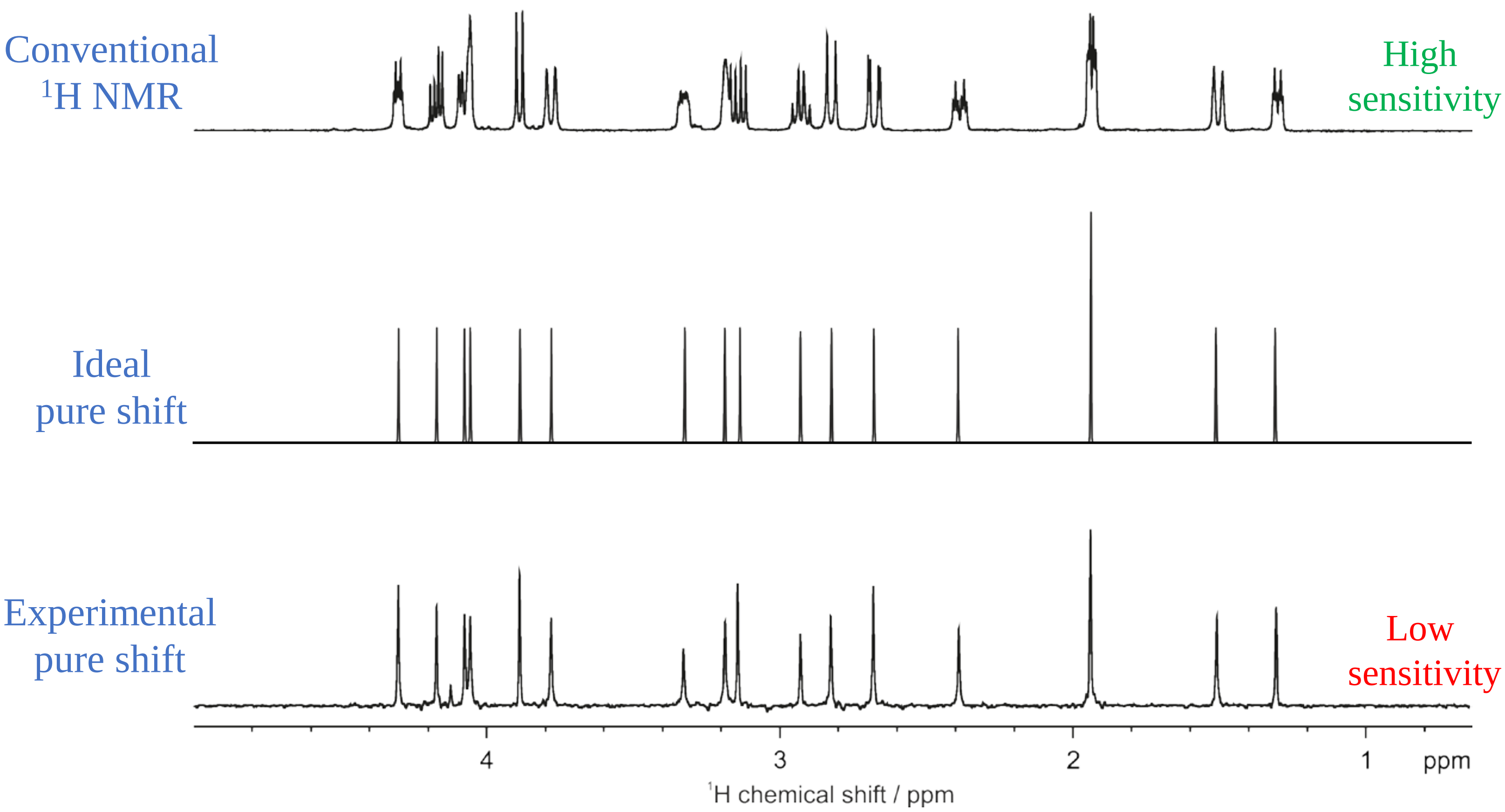
		Evolution during FID: δ	Evolution during FID: J_{HH}
Conventional ^{1}H NMR experiment		✓	✓
Ideal pure shift ^{1}H NMR experiment		✓	✗



Apply a **decoupling element** (few µs) after each acquisition point to:
- refocus J_{HH}
- leave δ to evolve

Not (currently) possible

How do we get a pure shift spectrum?

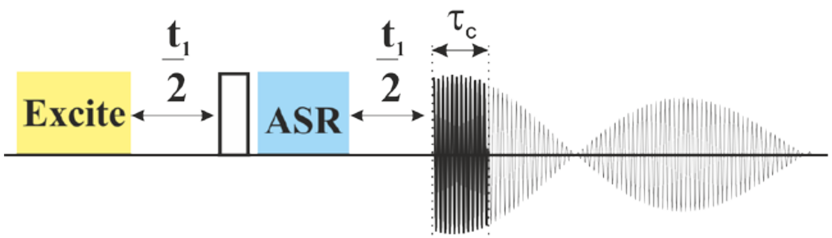


In molecular systems with homonuclear couplings, to get **a perfect pure shift spectrum is an unattainable ideal**: all we can do is to approximate it as closely as possible.

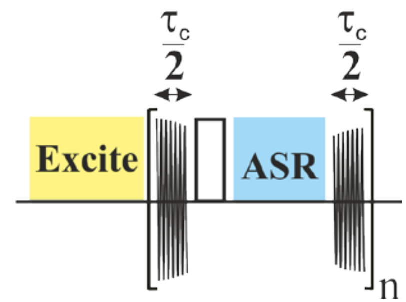
How do we get a pure shift FID?

Acquisition mode

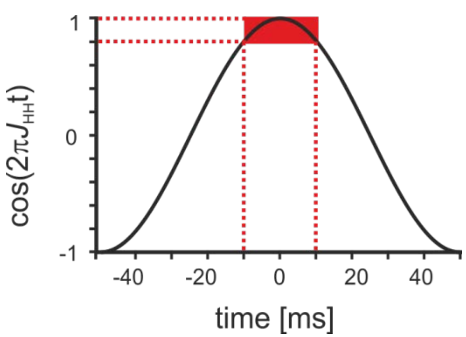
Interferogram



Real-time



Chunking data acquisition approach



J_{HH} = 10 Hz

$1/J_{HH}$ = 100 ms

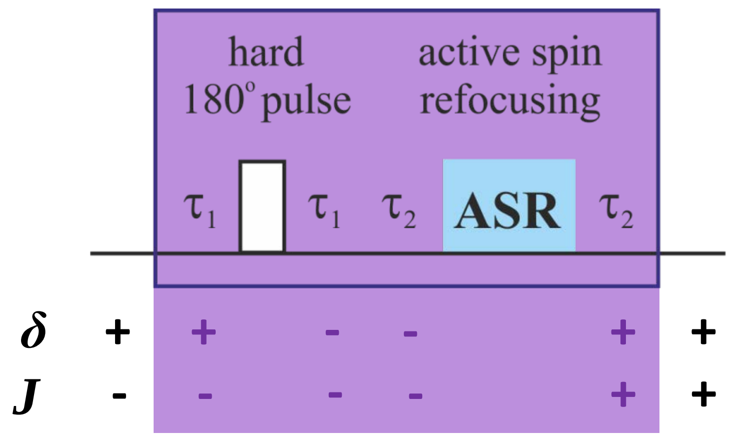
⬇

chunk duration (τ_c)

$\tau_c \approx$ 20 ms

$J \ll \delta$ allows data acquisition
in 'chunks' lasting $\ll 1/J_{HH}$
(J-evolution during the chunk is negligible)

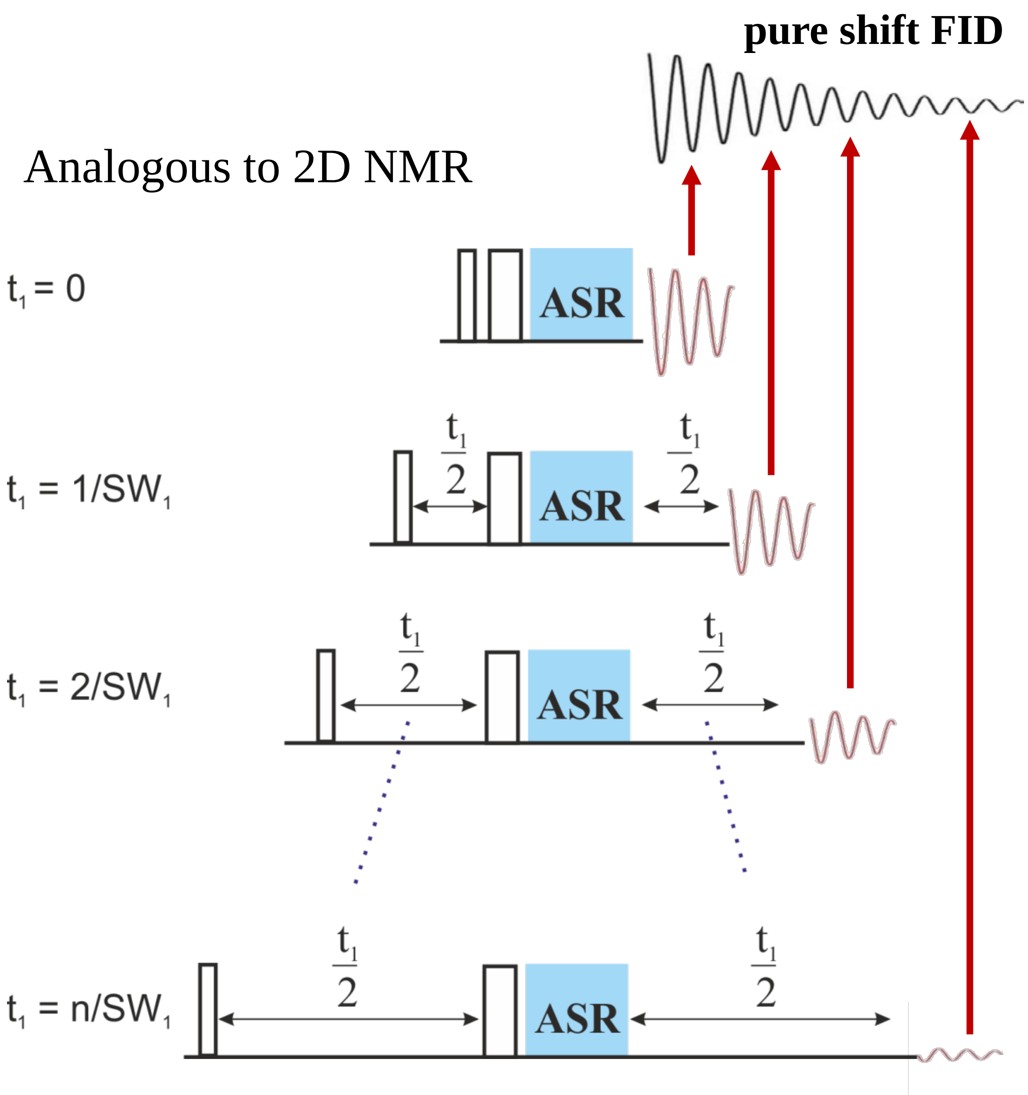
"*J*-refocusing" element



Separate the effects of shifts (δ) and couplings (J_{HH})

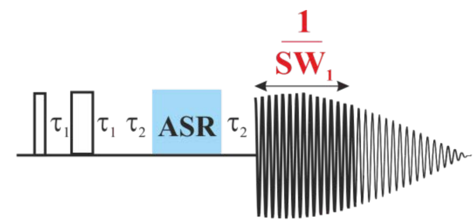
Interferogram pure shift NMR experiments – 2D acquisition

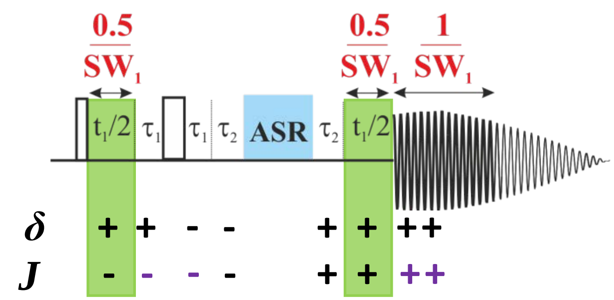
Analogous to 2D NMR



Increment in t_1 delay ($1/SW_1$) is set to the duration of the chunk

$\tau_c = 1/SW_1$



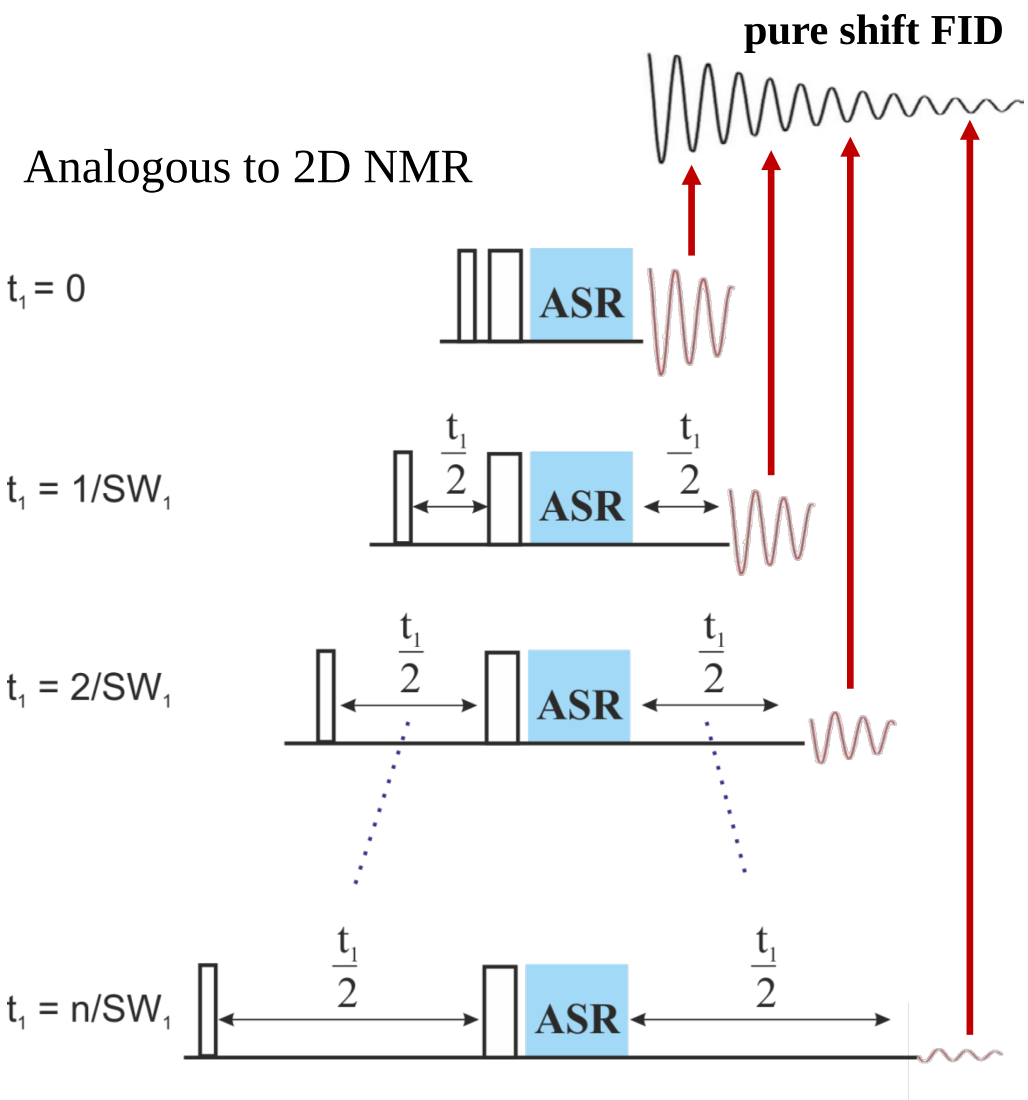


During t_1 δ evolves, but J is refocused

J. Magn. Reson. **124**, 486 (1997); *Angew. Chem. Int. Ed.* **49**, 3901 (2010)

Interferogram pure shift NMR experiments – 2D acquisition

Analogous to 2D NMR



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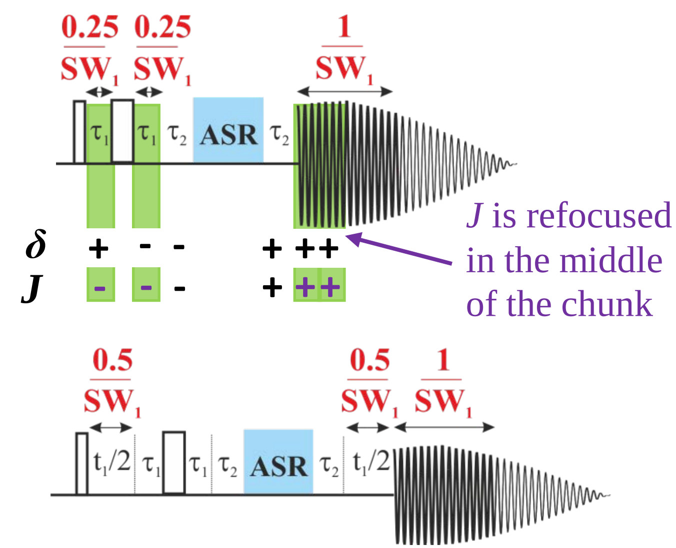


τ_1 is normally set to ensure that J is refocused at the midpoint of each chunk

Speeding things up

Real-time approach

a single experiment

Interferogram approach

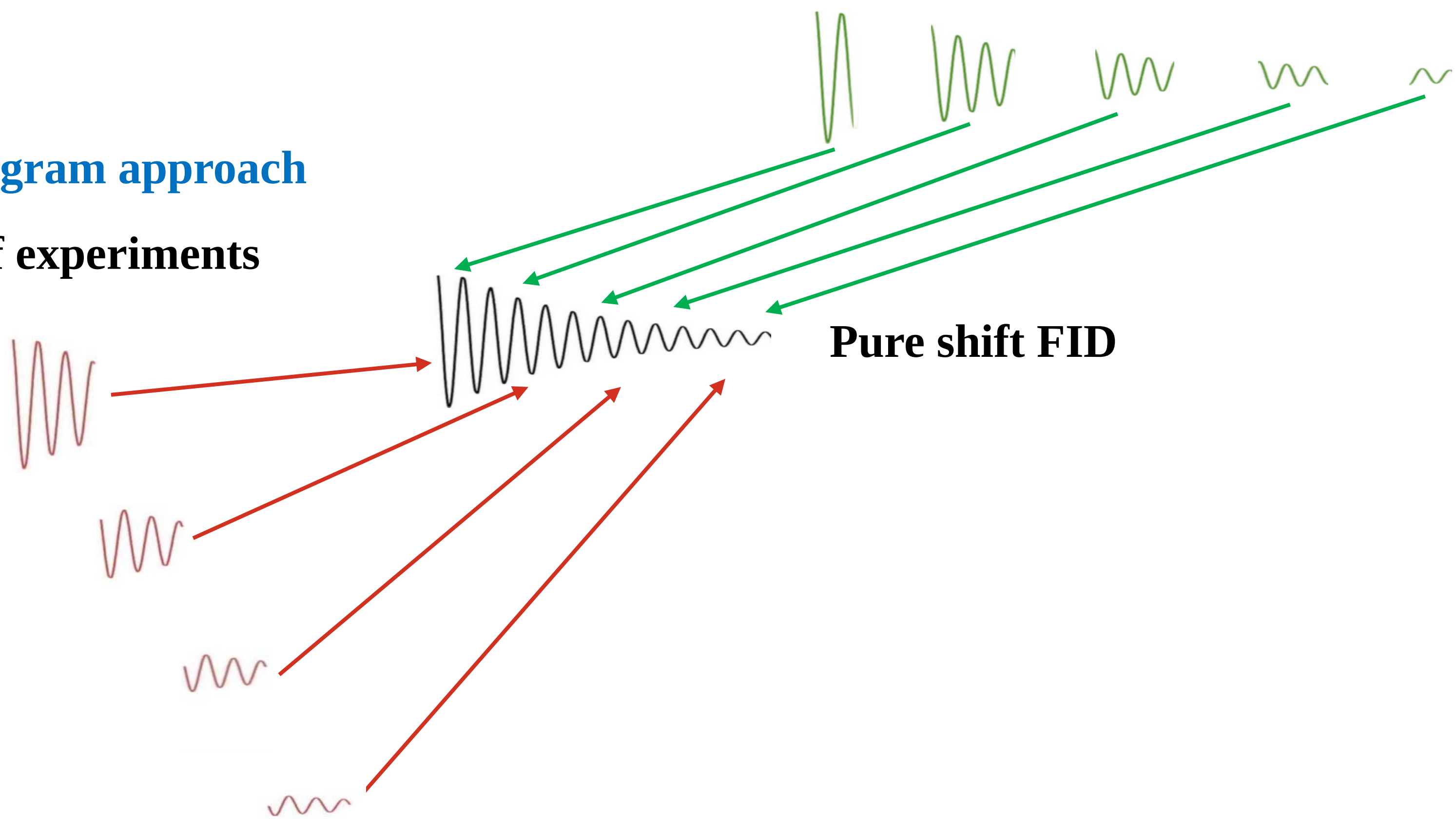
a set of experiments

1st experiment

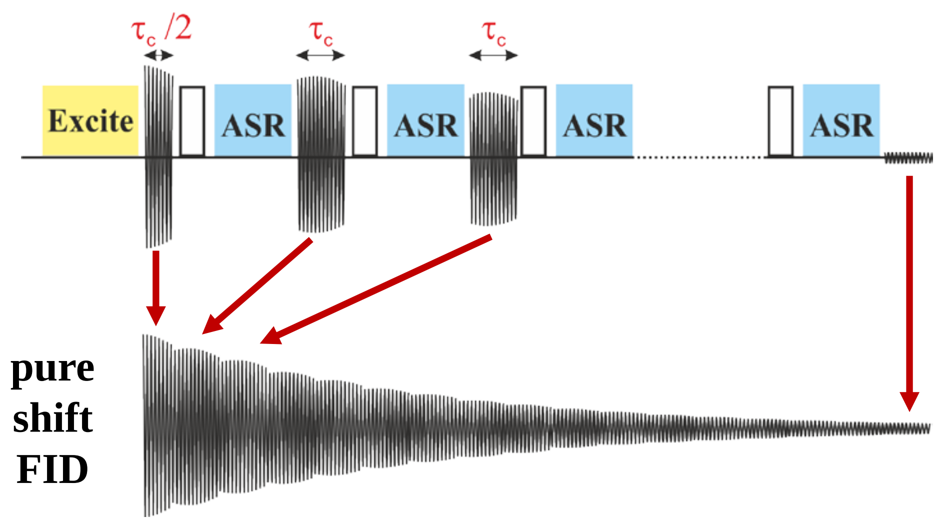
2nd experiment

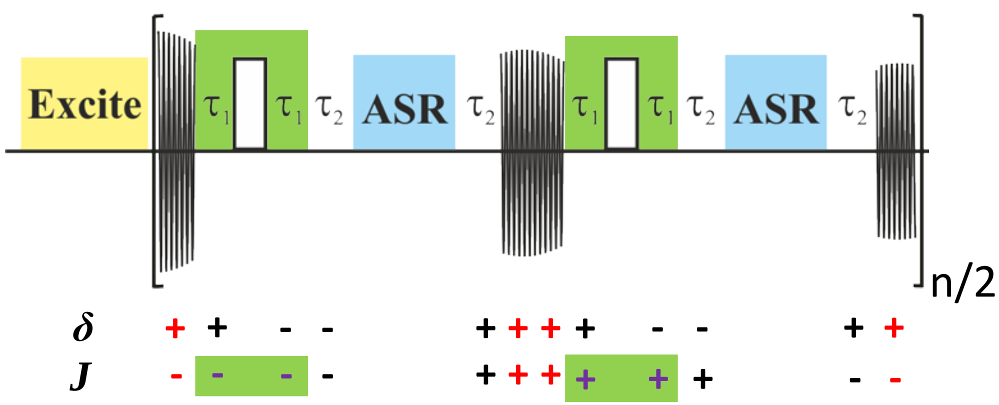
3rd experiment

4th experiment



Real-time pure shift NMR experiments – 1D acquisition





Chunks are collected from a single scan

The number of chunks (n) depends on the desired chunk duration (τ_c) and acquisition time (AQ):

$$n = \tau_c \, AQ$$

The first chunk is typically of half duration compared with all the others

τ_1 should ideally be zero to ensure that *J* is refocused at the midpoint of each chunk (except the first one)

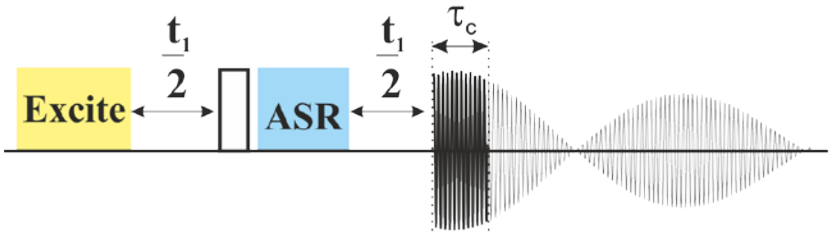
If not, *J* is refocused at the midpoint of every other chunk

J. Magn. Reson. **218**, 141 (2012)

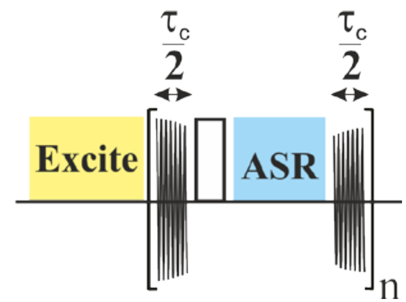
How do we get a pure shift FID?

Acquisition mode

Interferogram



Real-time



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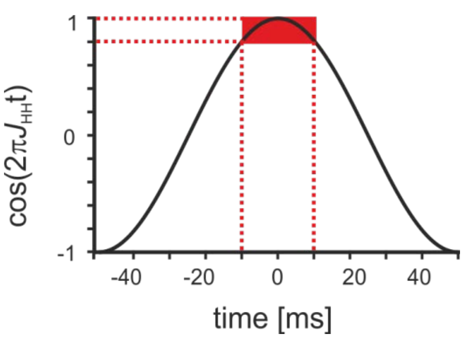

J_{HH} = 10 Hz

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⬇

chunk duration (τ_c)

$\tau_c \approx 20$ ms

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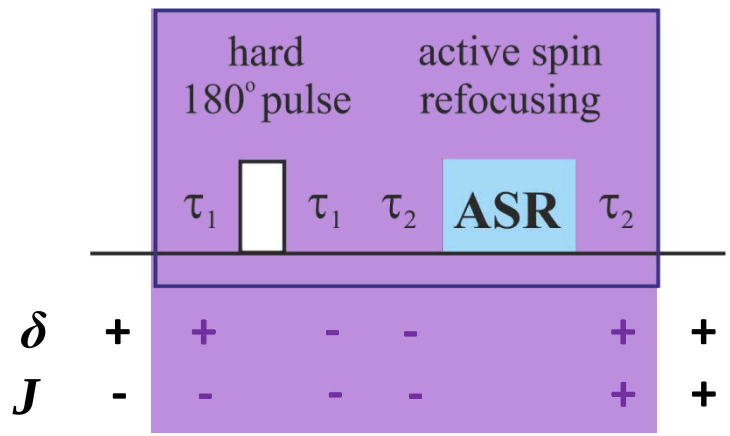
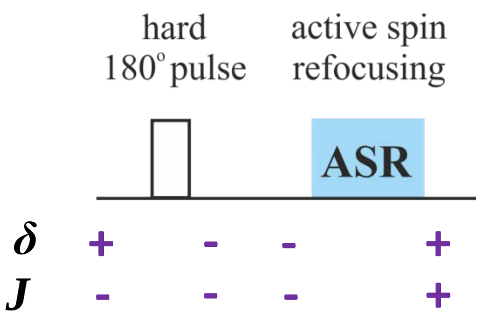


Separate the effects of shifts (δ) and couplings (J_{HH})

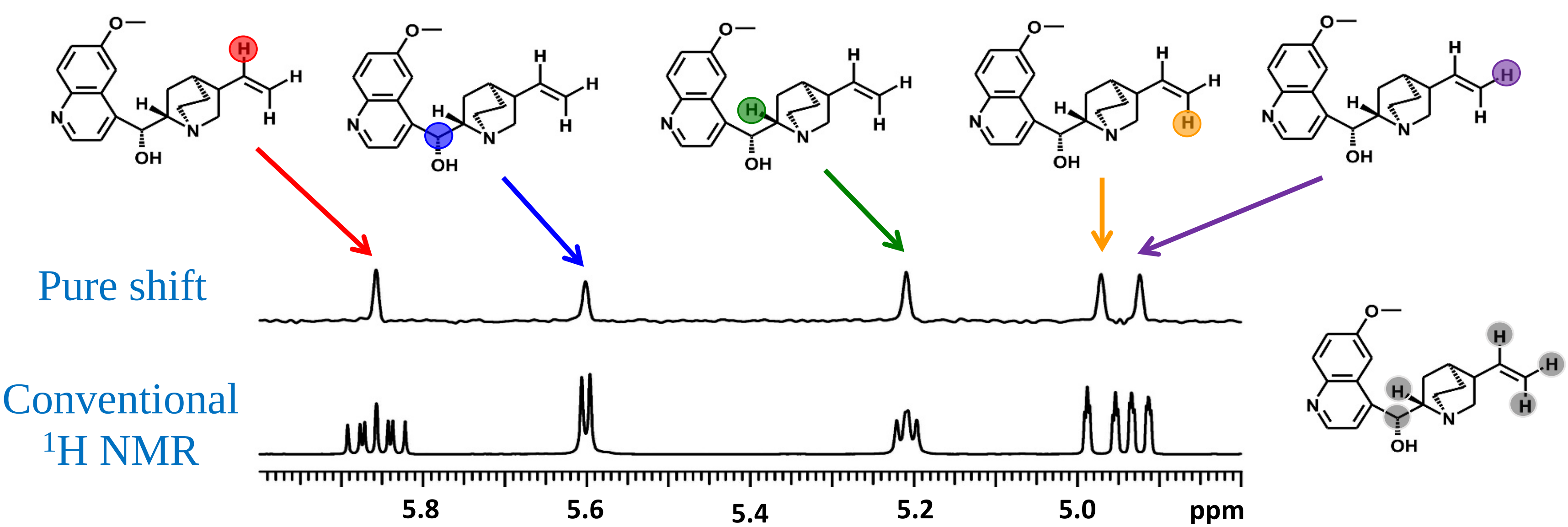
General mechanism for *J*-refocusing



Separate the effects of shifts (δ) and couplings (J_{HH})

- Hard 180° pulse: reverses effects of ***δ*** but not of ***J***
- ASR: reverses both ***δ*** and ***J***, **but for active spin only**, leaving passive spins unperturbed

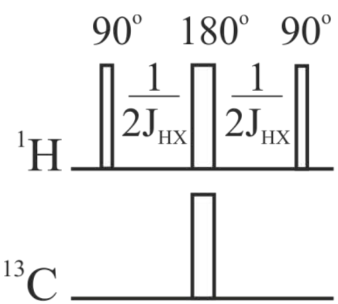
The concept of active and passive spins



Active spin refocusing methods

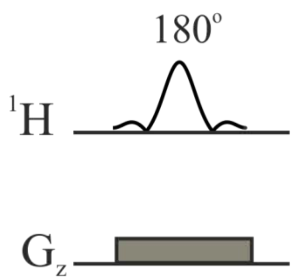
The “active spin refocusing” element divides the available spins into ***active*** spins that we observe, and ***passive*** spins that we manipulate to suppress the effects of couplings

BIRD



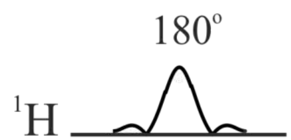
180° rotation of protons coupled to ^{13}C

Zangger-Sterk (ZS)



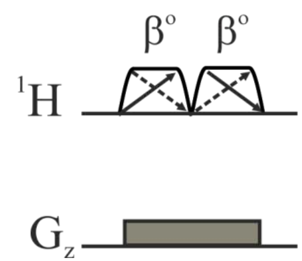
Slice and shift selective 180° rotation

Band-selective (BS)



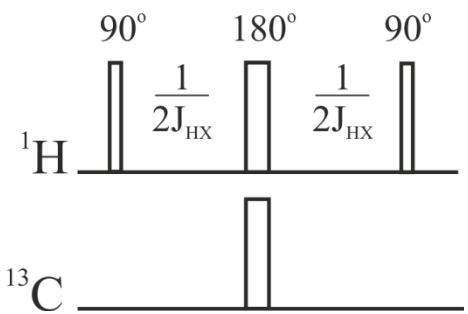
shift selective 180° rotation

PSYCHE

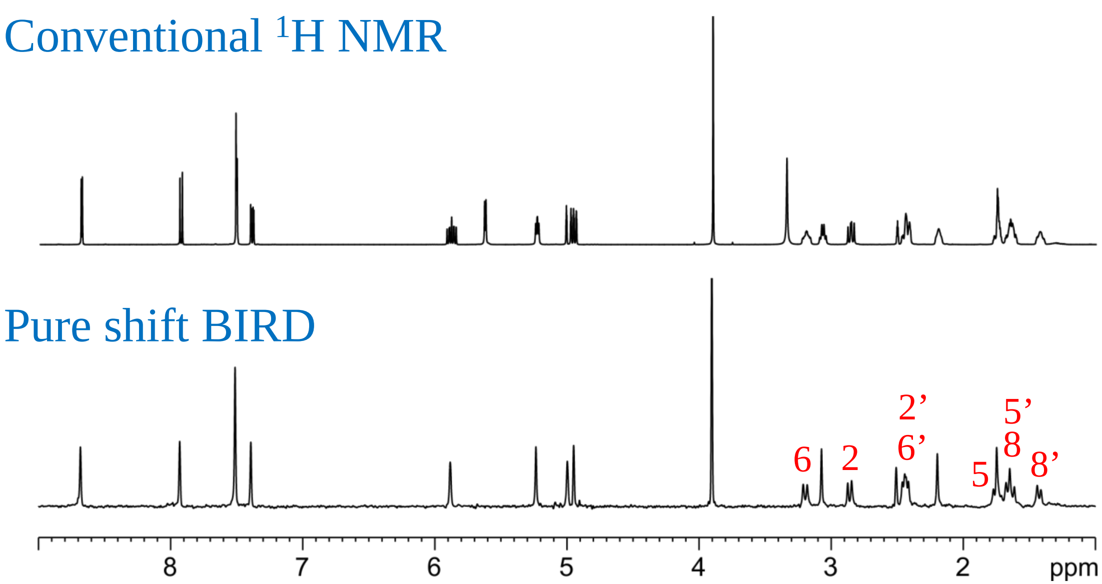


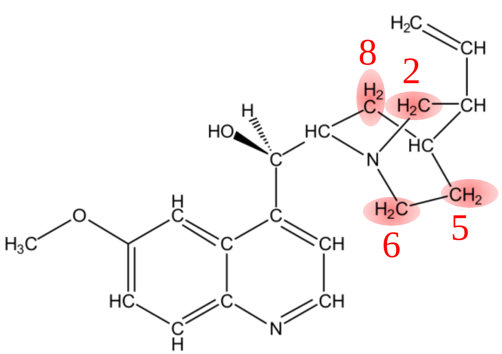
Flip a fraction $\sin^2\beta$ of spins

Bilinear rotation decoupling (BIRD)

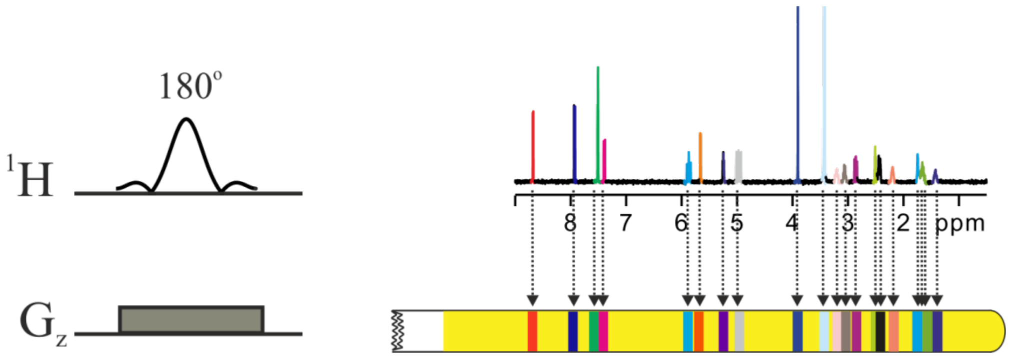


- BIRD$_d$ inverts only protons directly coupled to ^{13}C (or ^{15}N)
- Isotopic dilution ensures that their coupled partners are not inverted
- Protons are *active* if attached to ^{13}C (or ^{15}N), *passive* if not
- Compatible with both real-time and interferogram acquisition
- In molecules with natural abundance, sensitivity is limited by 1.1 % ^{13}C (0.37 % in ^{15}N)
- Protons attached to the same ^{13}C are not decoupled from one another – geminal protons appear as doublets

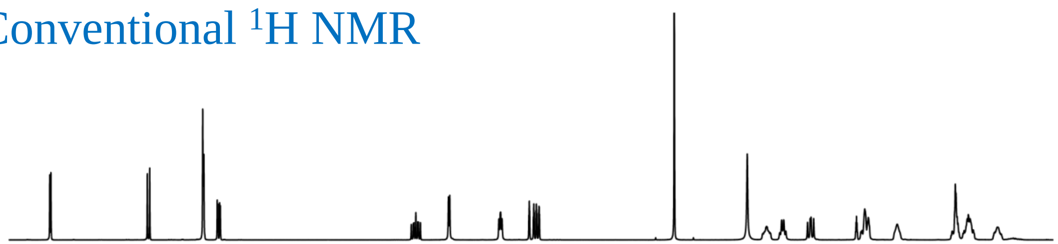




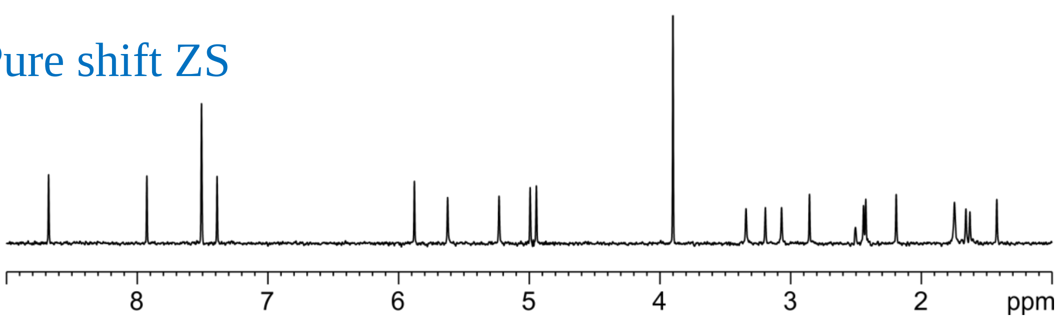
Zangger-Sterk (ZS)



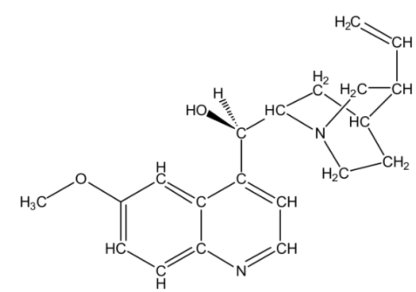
Conventional ^{1}H NMR



Pure shift ZS

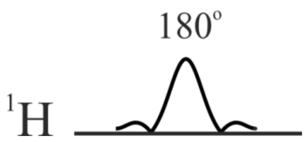


- Simultaneous application of a selective 180° pulse and a weak pulse field gradient
- Slice and shift selection
- Each *active* spin is excited in a narrow region (slice) of the sample
- Compatible with both real-time and interferogram acquisition
- Low sensitivity, proportional to the slice thickness (0.5-10 %)

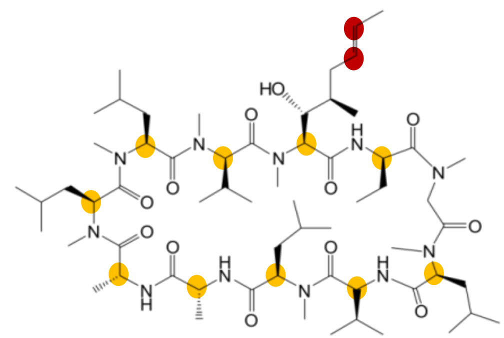


J. Magn. Reson. **124**, 486 (1997)

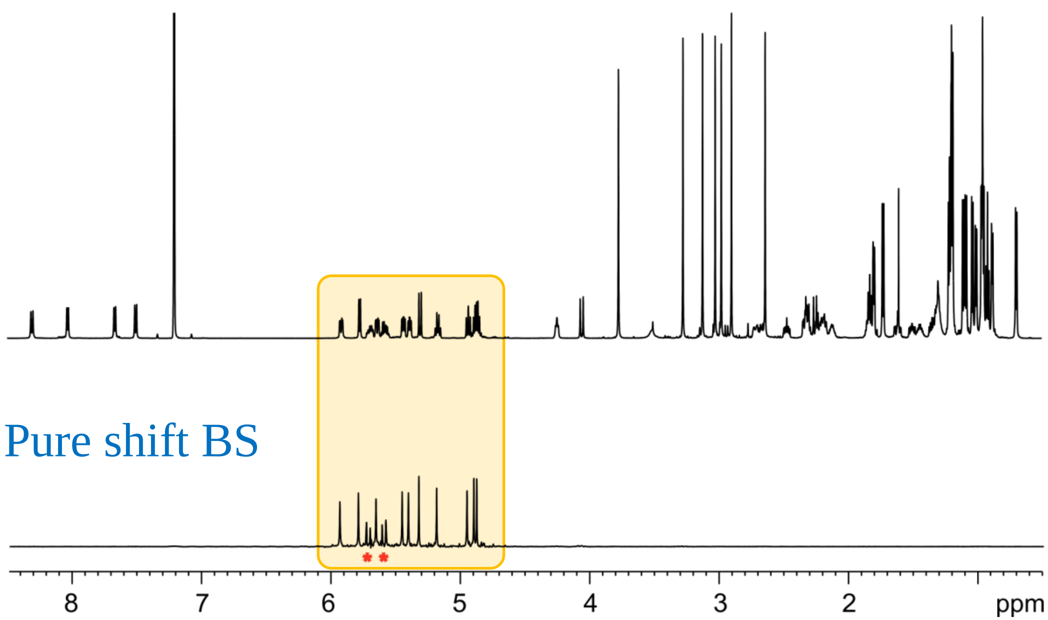
Band-selective (BS) – HOBS, BASH and BASHD

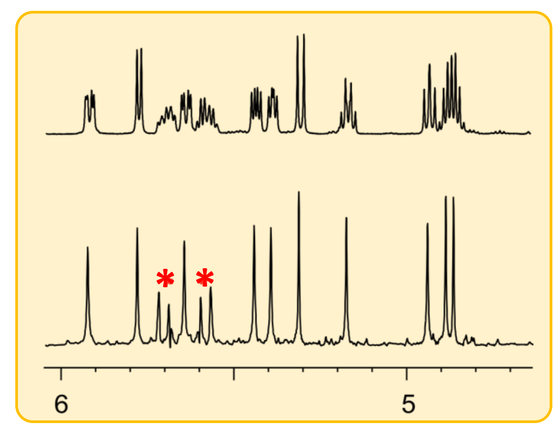


- The selective 180° pulse inverts only protons within its bandwidth (*active* spins)
- One (frequency selection) or several resonances (band selection or multiple-frequency selection) can be homodecoupled
- Need to avoid exciting coupled protons
- Compatible with both real-time and interferogram acquisition
- Excellent sensitivity (≥ 100 %)



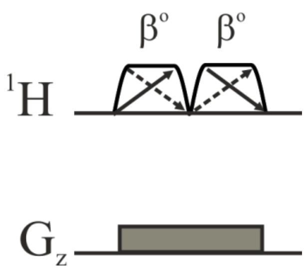
Conventional ^{1}H NMR





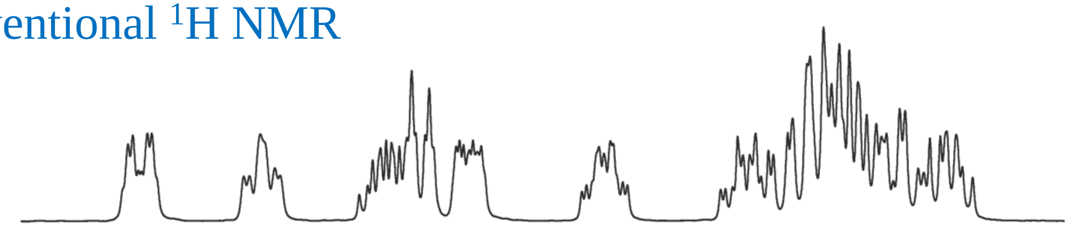
Chem. Eur. J. **19**, 17283 (2013); *J. Magn. Reson.* **241**, 97 (2014); *Chem. Commun.* **50**, 25127 (2014)

Pure shift yield by chirp excitation (PSYCHE)

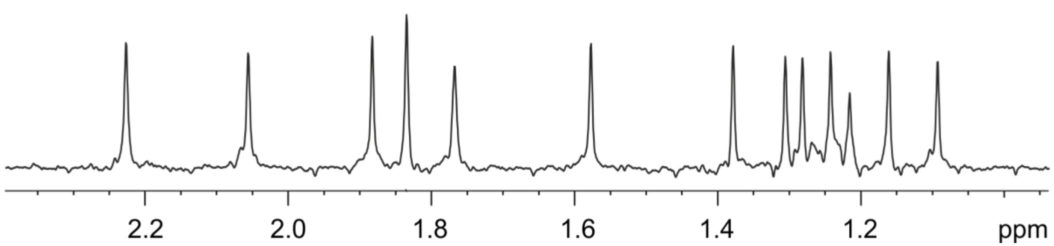


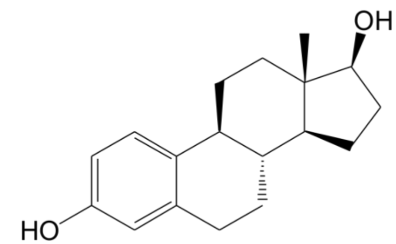
- Simultaneous application of a low-flip angle (β) saltire chirp pulse and a weak pulse field gradient
- Simplification of coupling patters using low flip angle pulses (similar to anti-z-COSY experiment)
- Only a fraction $\sin^2\beta$ of spins are inverted (statistical excitation)
- Sensitivity depends on $\sin^2\beta$ (3-20 %)
- Only compatible with interferogram acquisition

Conventional ^{1}H NMR



Pure shift PSYCHE



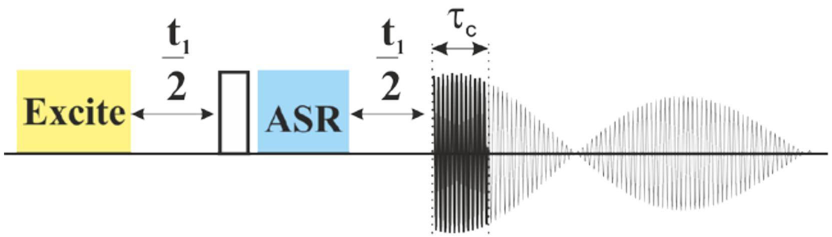


Angew. Chem. Int. Ed. **53**, 6990 (2014)

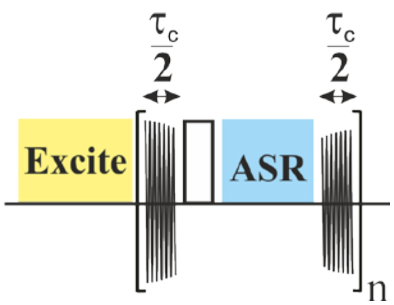
How can we use all these elements?

Acquisition mode

Interferogram

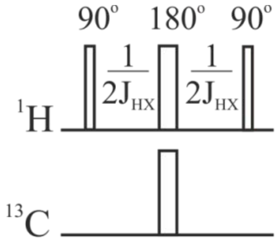


Real-time

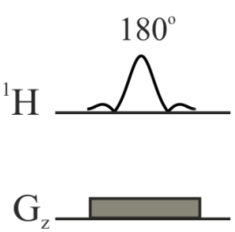


Active spin refocusing methods

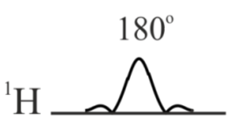
BIRD



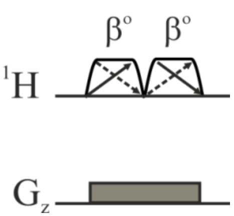
ZS



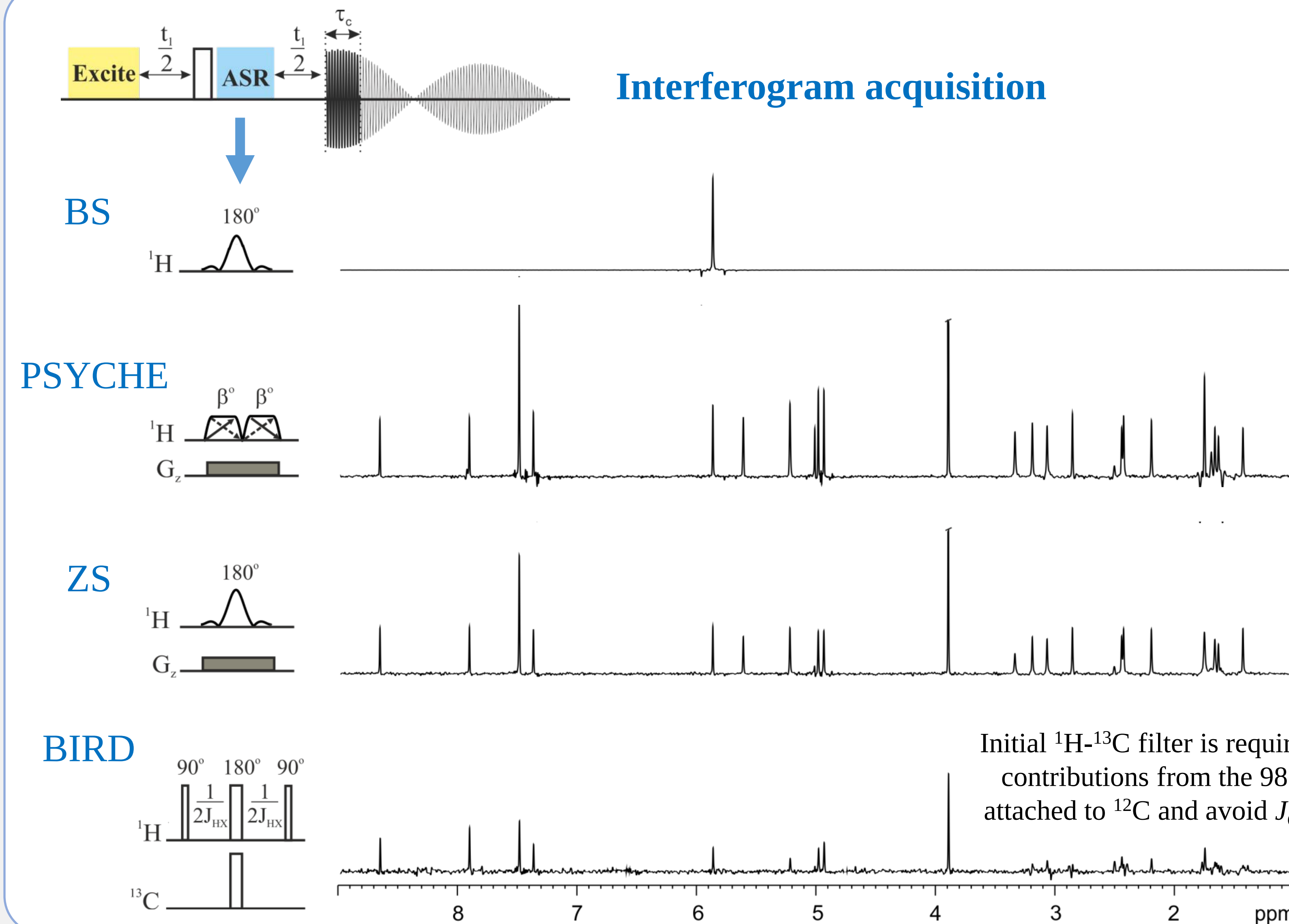
BS



PSYCHE

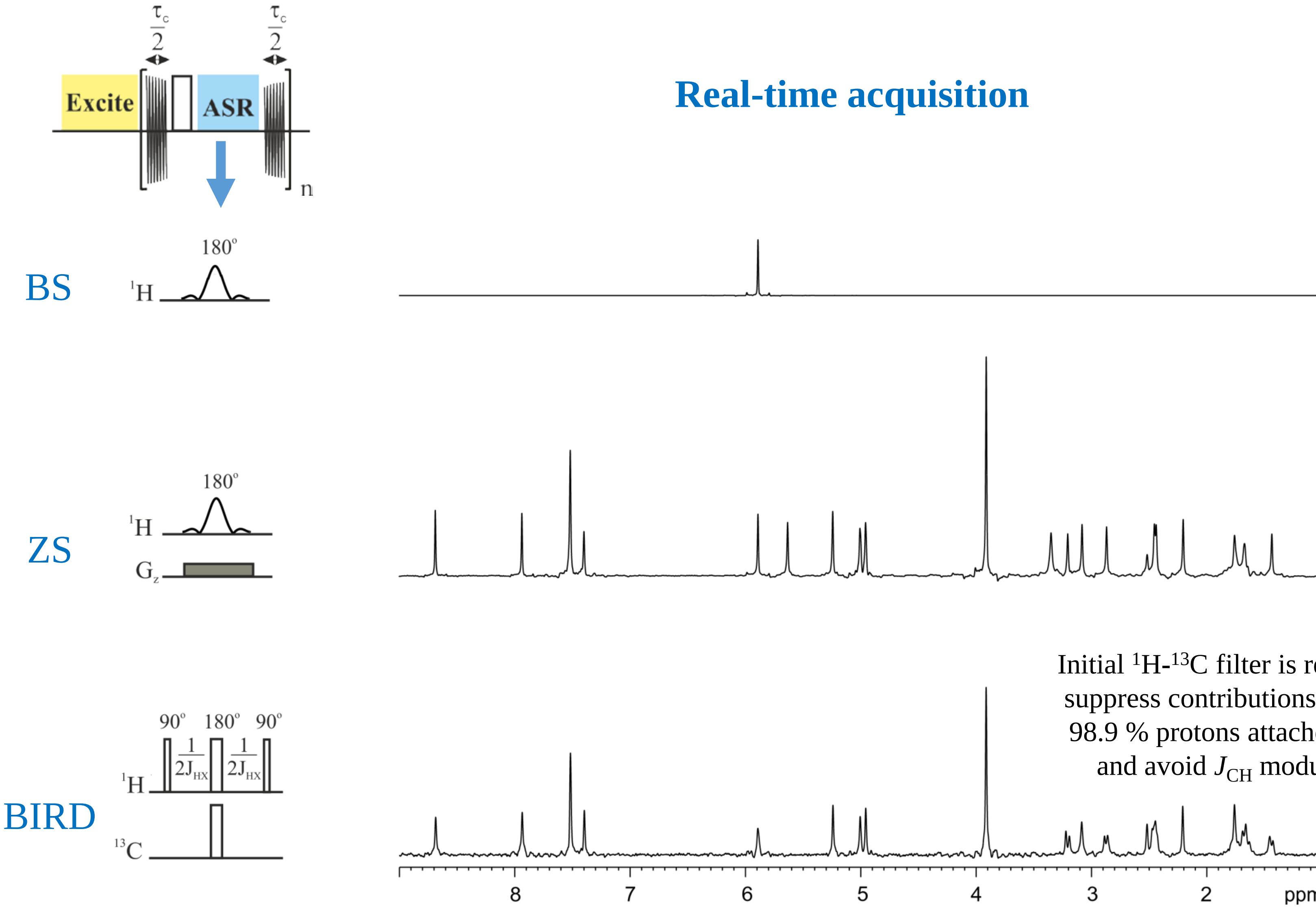


Combining ASR elements and interferogram acquisition



Initial ^{1}H-^{13}C filter is required to suppress contributions from the 98.9 % protons attached to ^{12}C and avoid J_{CH} modulation

Combining ASR elements and real-time acquisition



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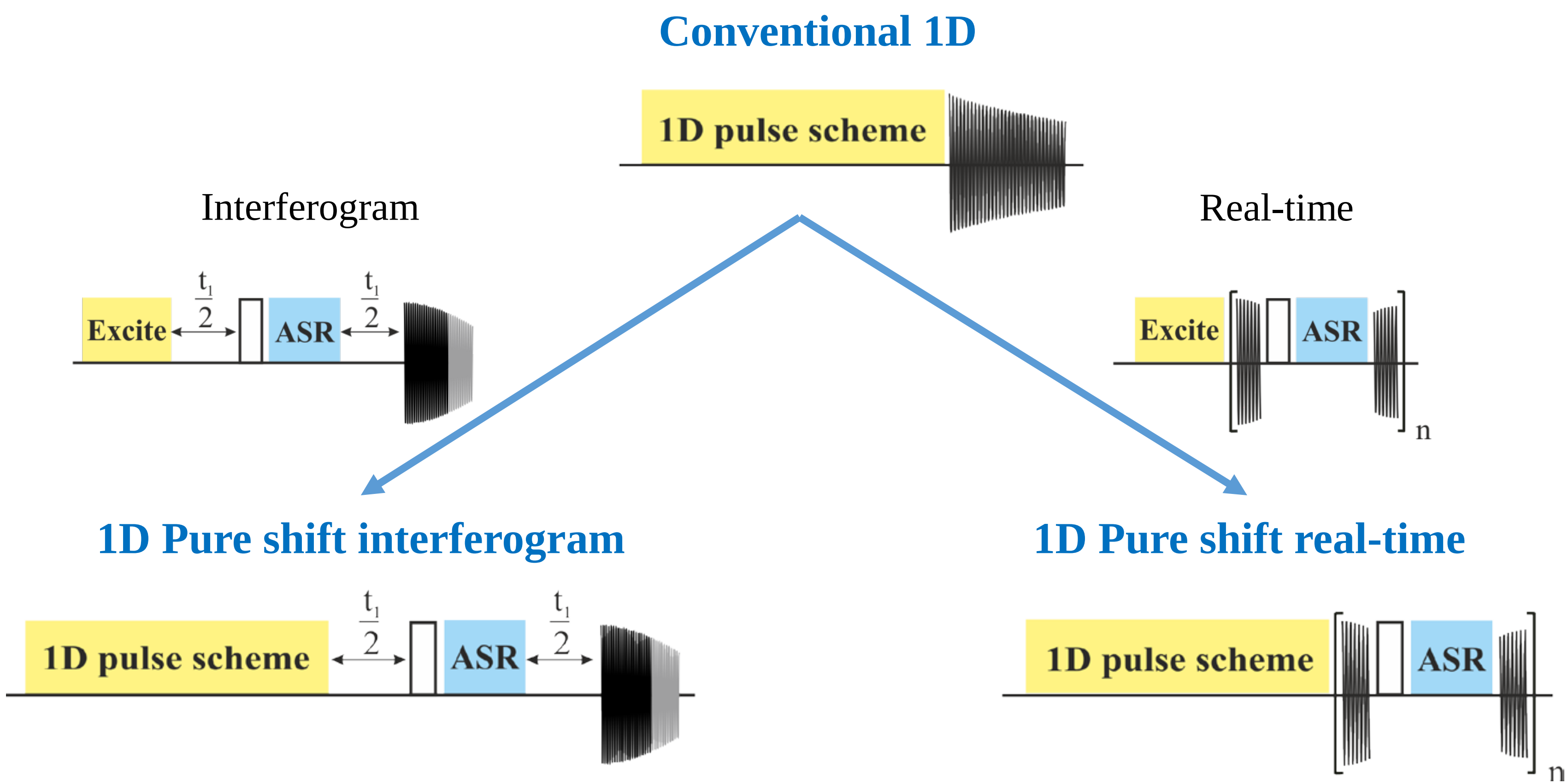
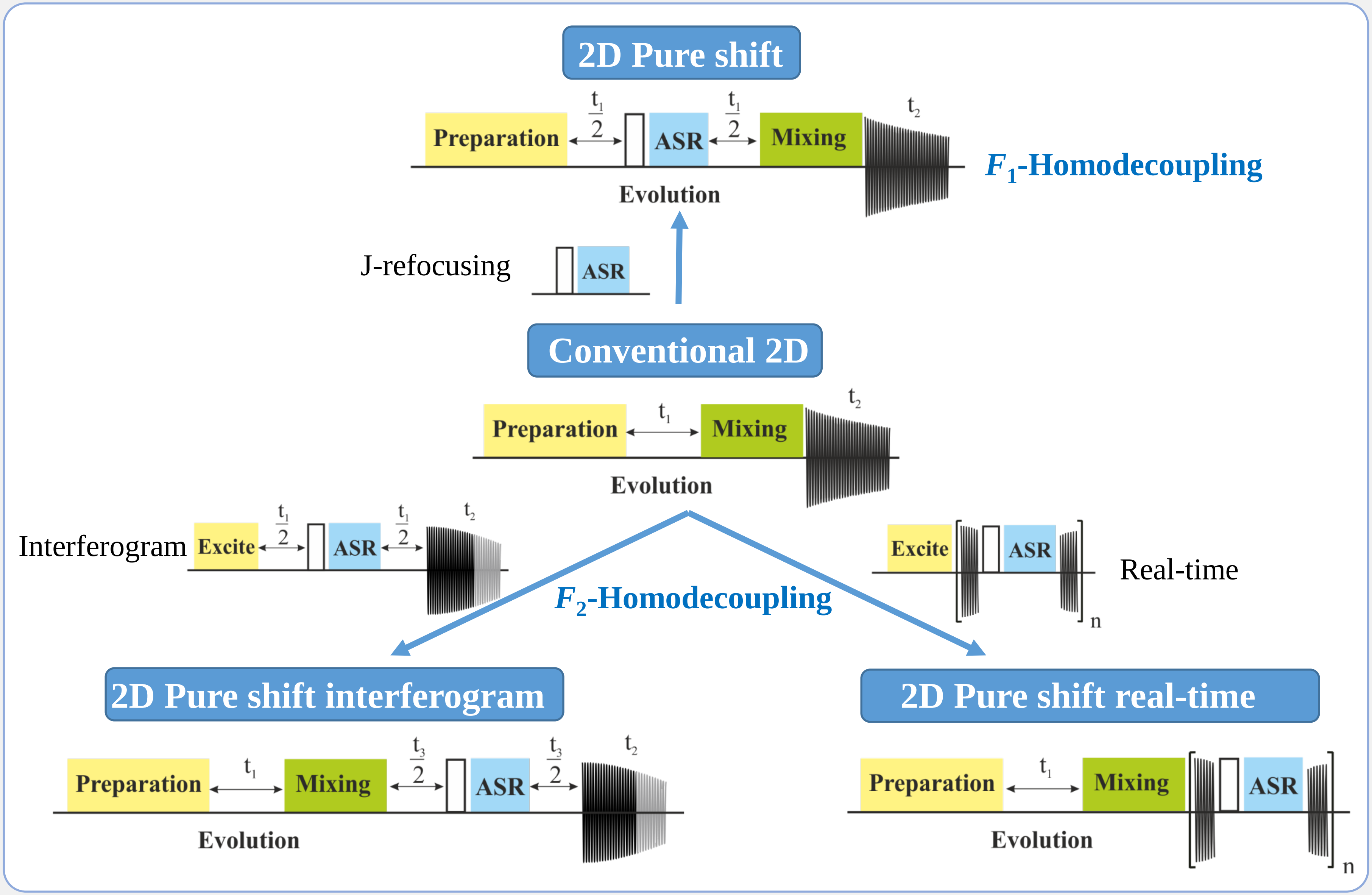
Implementing pure shift methods in 1D experiments: schematic representation



1D pulse scheme: Inversion recovery, CPMG / PROJECT, selective TOCSY, selective NOESY, etc.

Implementing pure shift methods in 2D experiments: schematic representation



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III - Practical aspects:

Sensitivity

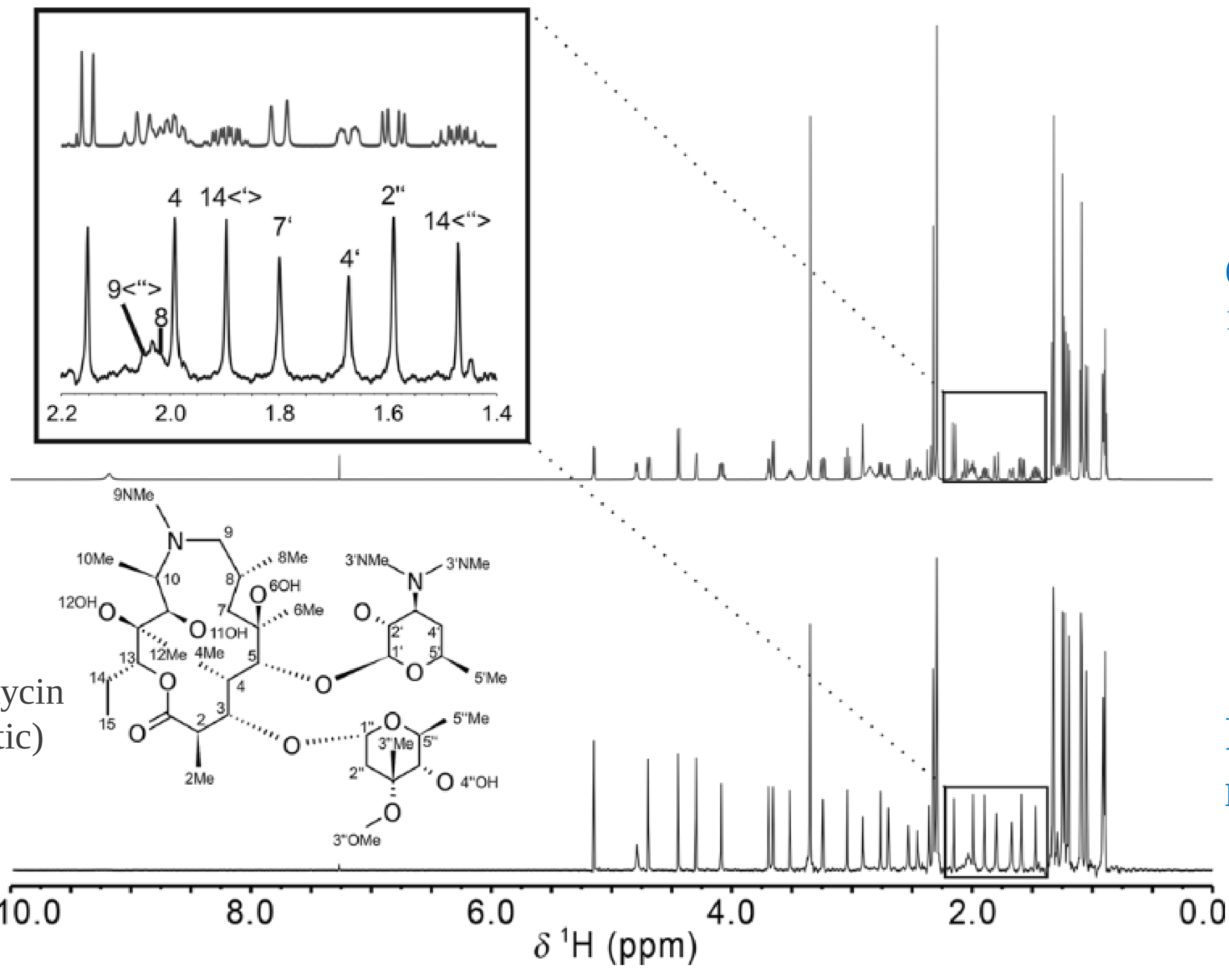
Spectral quality

Others

Review applications:
Magn. Reson. Chem. **53**, 399 (2015)

1D pure shift NMR experiments for structure analysis of small and medium sized molecules

Azithromycin
(antibiotic)



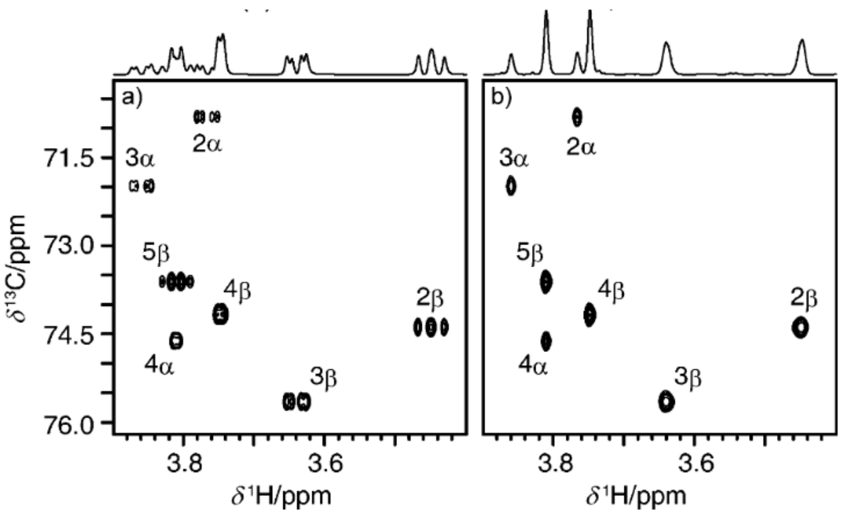
Conventional
^{1}H NMR

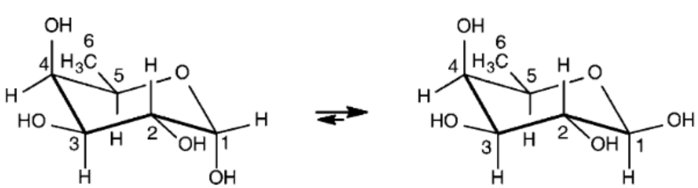
Pure shift
real-time ZS

Angew. Chem. Int. Ed. **52**, 7143 (2013)

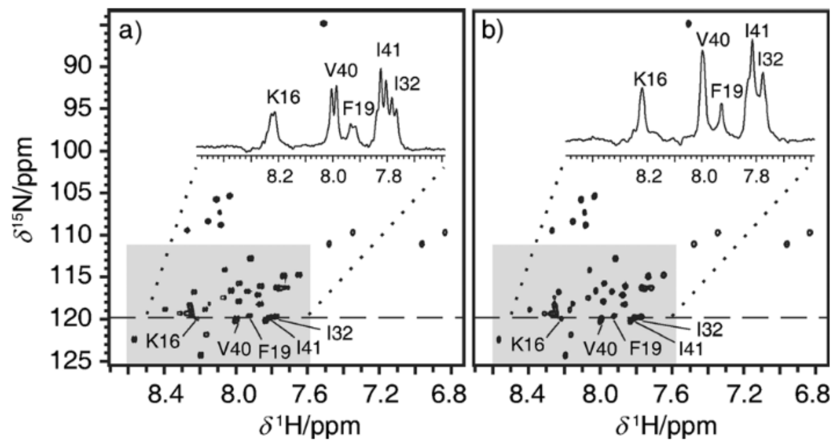
Heteronuclear 2D pure shift NMR experiments for structure analysis of small and medium sized molecules

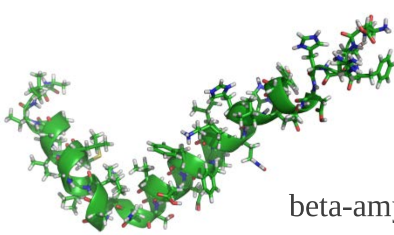
Conventional ^{1}H-^{13}C HSQC | Pure shift real-time ^{1}H-^{13}C HSQC | Conventional ^{1}H-^{15}N HSQC | Pure shift real-time ^{1}H-^{15}N HSQC





α-D(+)-fucose β-D(+)-fucose



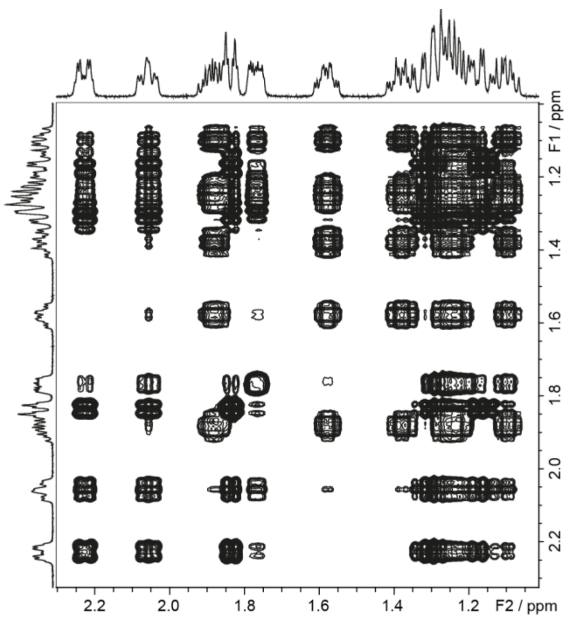


^{15}N-labeled beta-amyloid peptide 1-42 (Aβ)

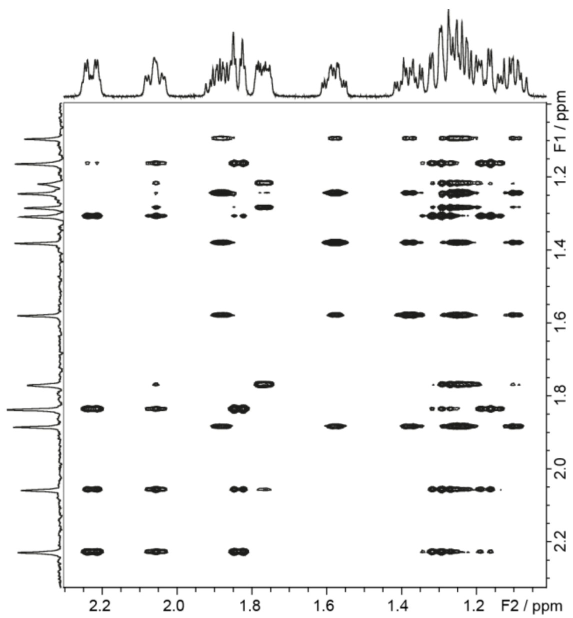
Angew. Chem. Int. Ed. **52**, 11616 (2013)

Homonuclear 2D pure shift NMR experiments for structure analysis of small and medium sized molecules

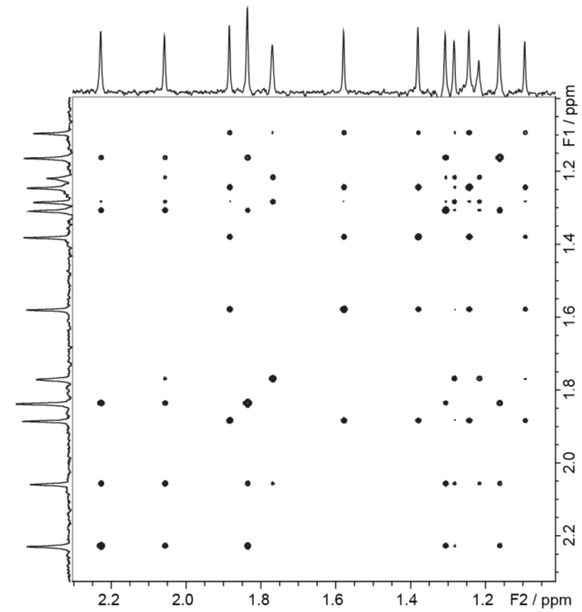
Conventional TOCSY

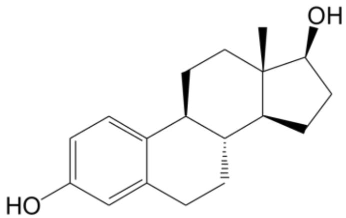


Pure shift F_1-PSYCHE TOCSY



Pure shift F_1-PSYCHE TOCSY and indirect covariance processing





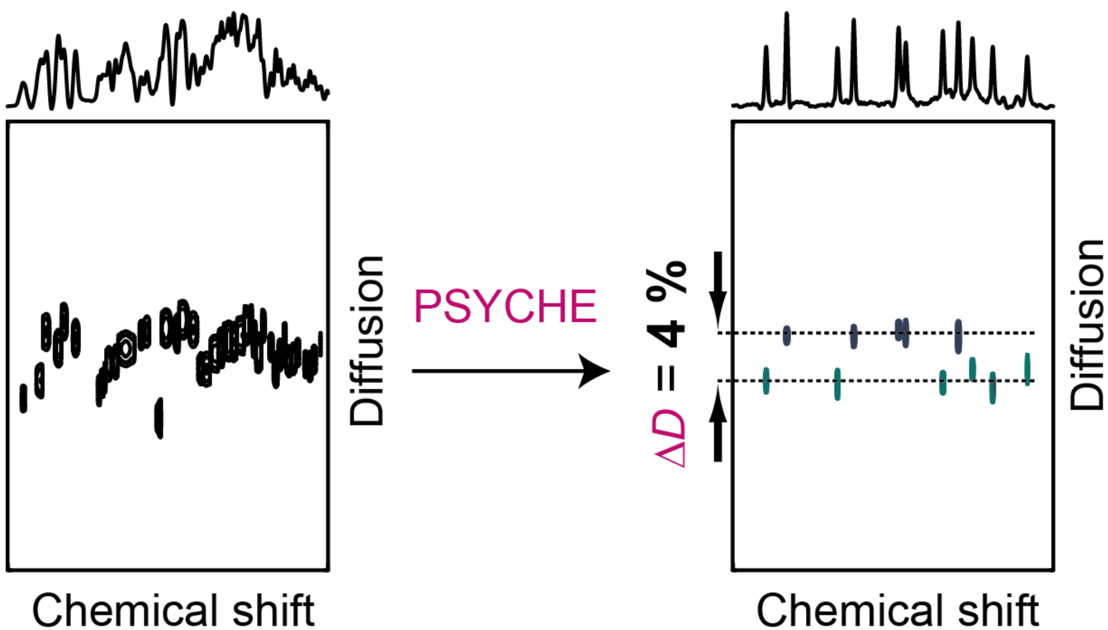
Estradiol (steroid)

J. Am. Chem. Soc. **136**, 11867 (2014)

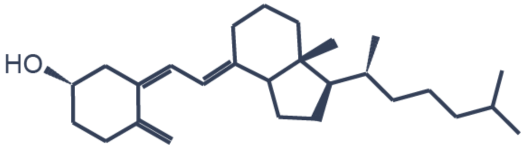
Ultrahigh-resolution diffusion-ordered spectroscopy

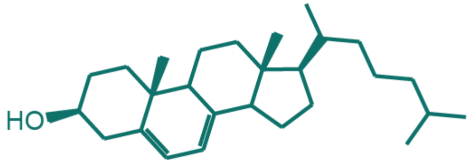
Conventional DOSY

Pure shift interferogram
PSYCHE-iDOSY



Vitamin D_3
(secosteroids)





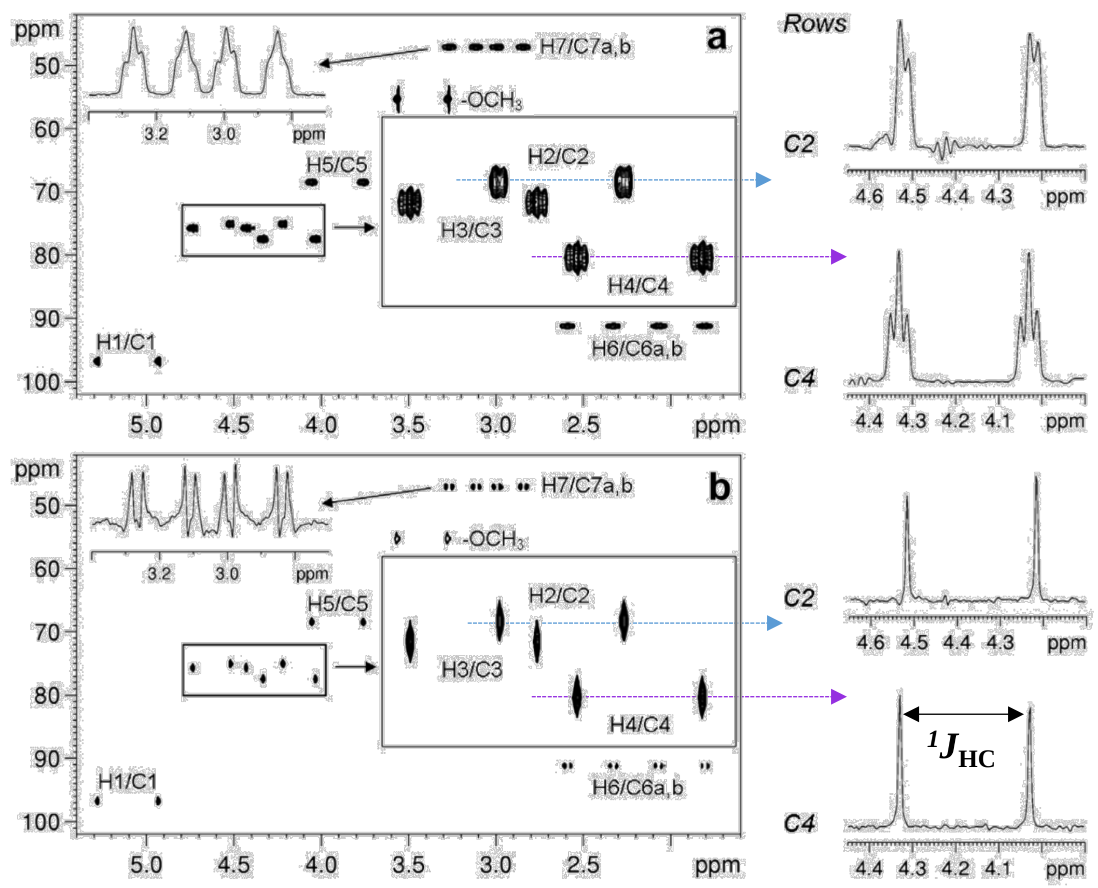
Provitamin D_3
(zoosterol)

Angew. Chem. Int. Ed. **55**, 15579 (2016)

2D Pure shift NMR experiments for accurate determination of one-bond heteronuclear coupling constants

Conventional
^{1}H-^{13}C CLIP-HSQC

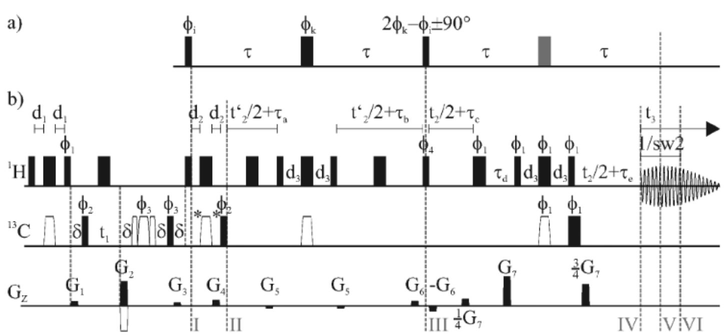
Pure shift
interferogram
^{1}H-^{13}C CLIP-HSQC

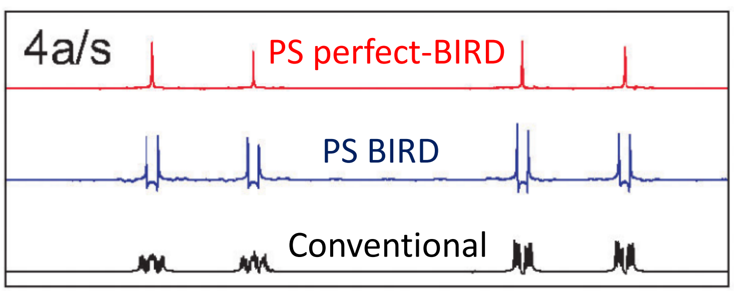


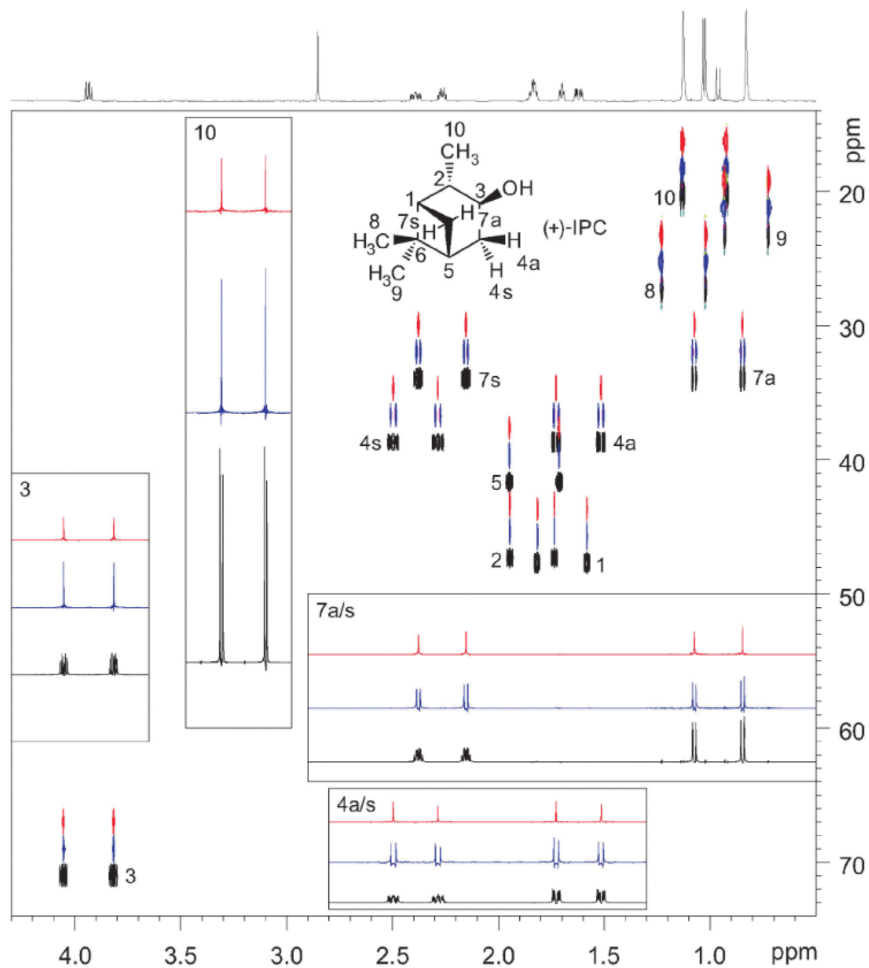
J. Magn. Reson. **239**, 110 (2014); *J. Magn. Reson.* **239**, 130 (2014)

2D Pure shift NMR experiments for accurate determination of one-bond heteronuclear coupling constants

Pure shift interferogram perfect-BIRD
^{1}H-^{13}C CLIP-HSQC





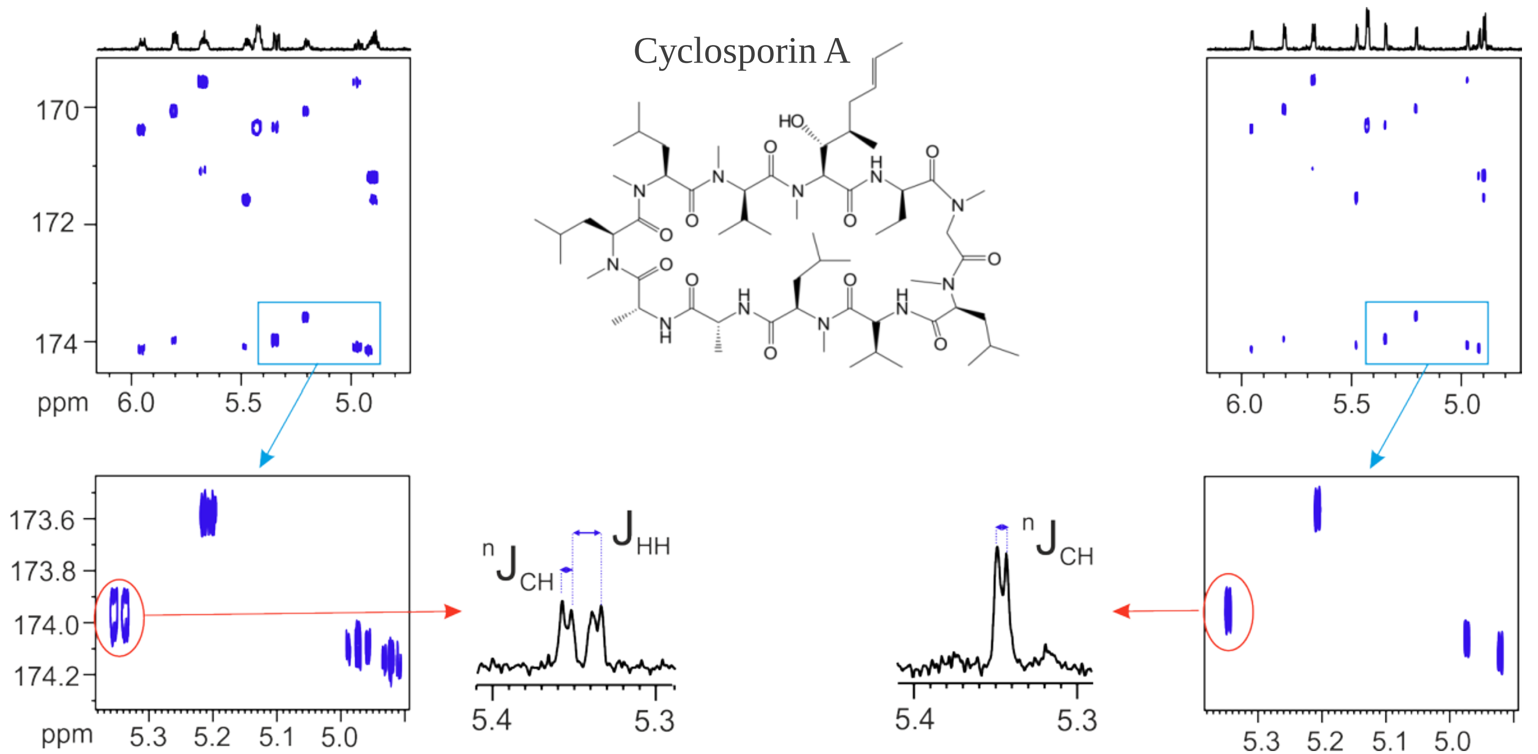


Chem. Commun. **50**, 15702 (2014)

2D Pure shift NMR experiments for accurate determination of long-range heteronuclear coupling constants

Conventional ^{1}H-^{13}C HSQMBC

Pure shift real-time BS ^{1}H-^{13}C HSQMBC

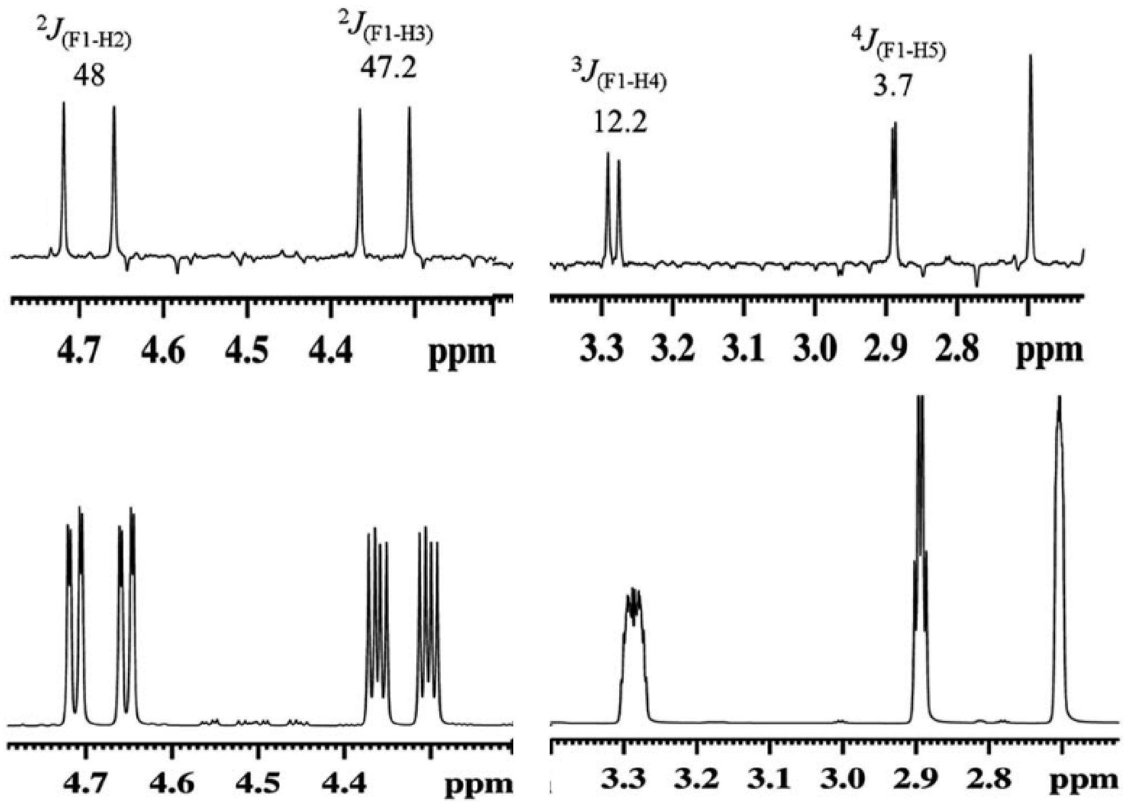


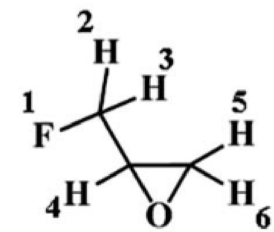
J. Magn. Reson. **238**, 63 (2014)

1D Pure shift NMR experiments for fast and accurate extraction of heteronuclear coupling constants

Pure shift interferogram ZS

Conventional ^{1}H NMR





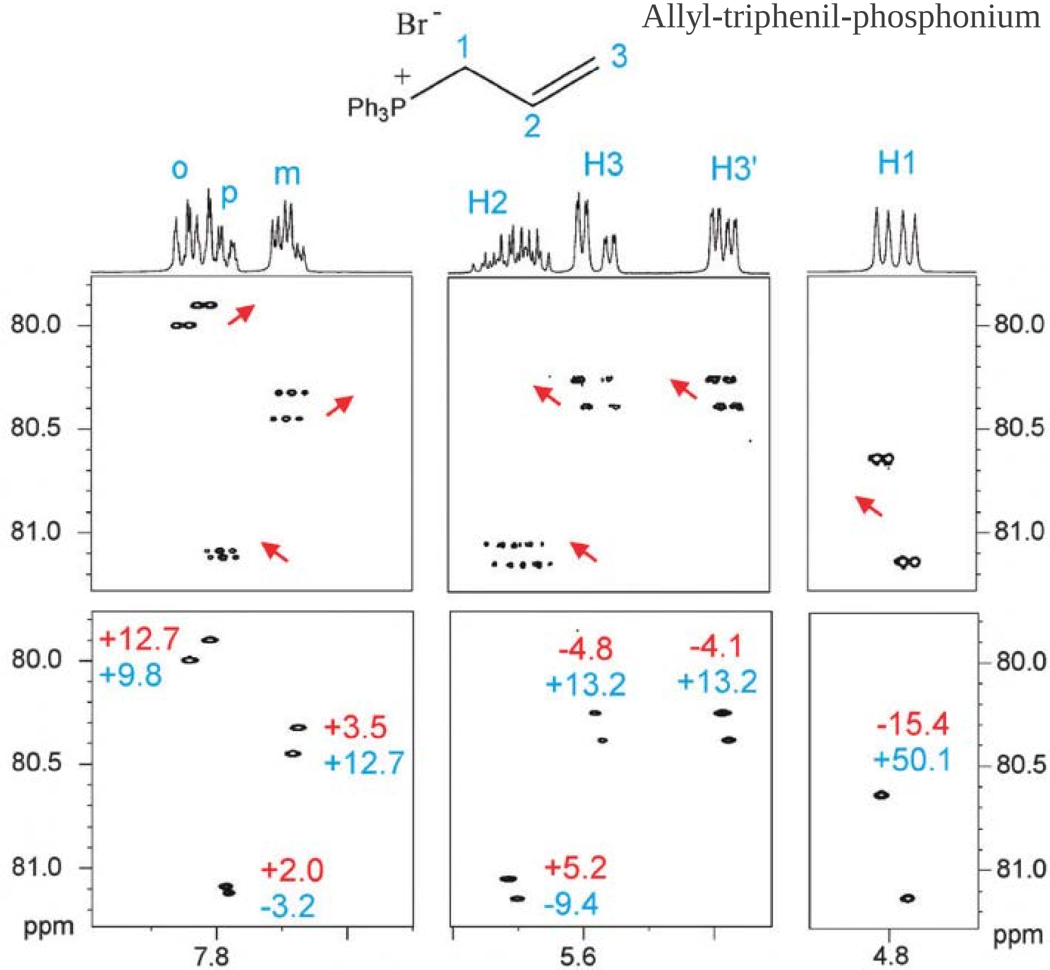
Epifluorohydrin

2D Pure shift NMR experiments for fast and accurate extraction of heteronuclear coupling constants

Allyl-triphenil-phosphonium bromide

Spectral-aliased 2D ^{1}H-^{13}C HSQC

Pure shift real-time spectral-aliased 2D ^{1}H-^{13}C HSQC



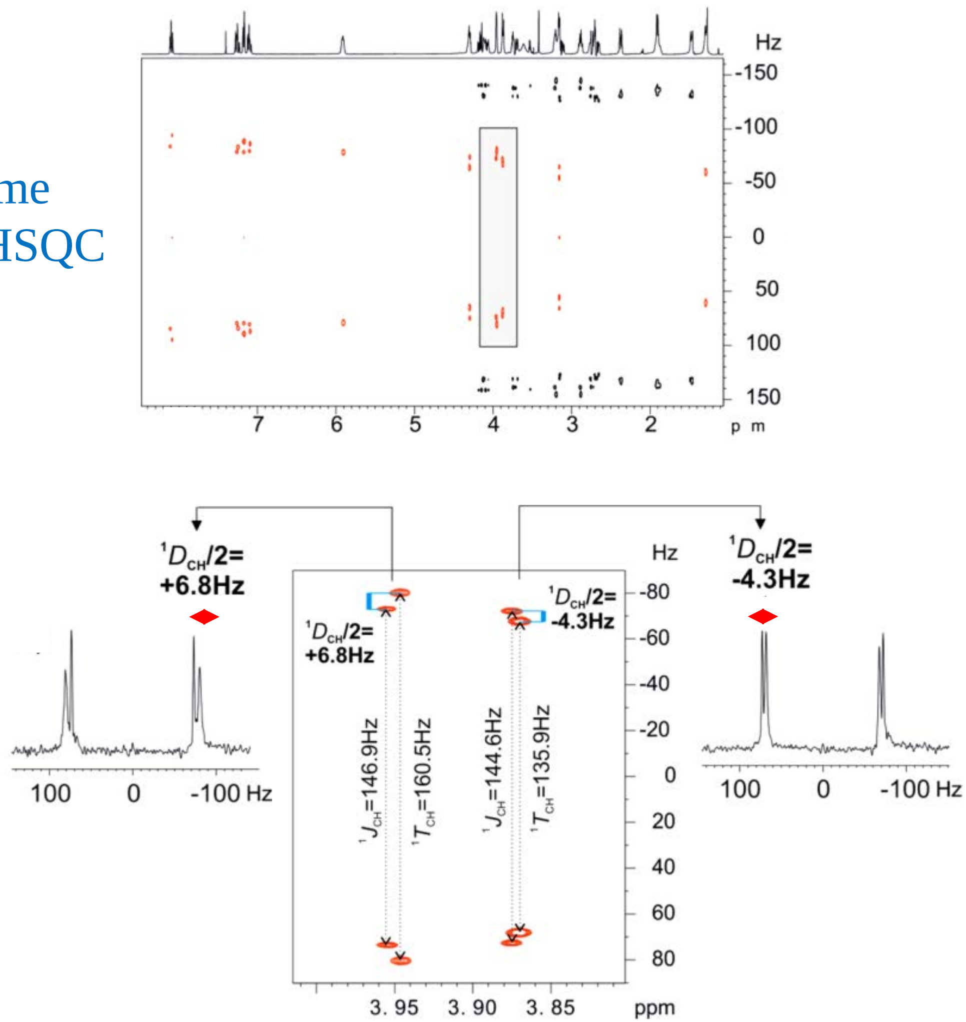
J_{HP}

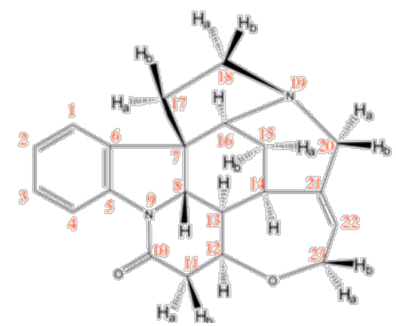
J_{CP}

Chem. Commun. **51**, 3262 (2015)

One-shot determination of residual dipolar coupling (RDCs) using pure shift NMR experiments

Pure shift real-time *J*-resolved ^{1}H-^{13}C HSQC





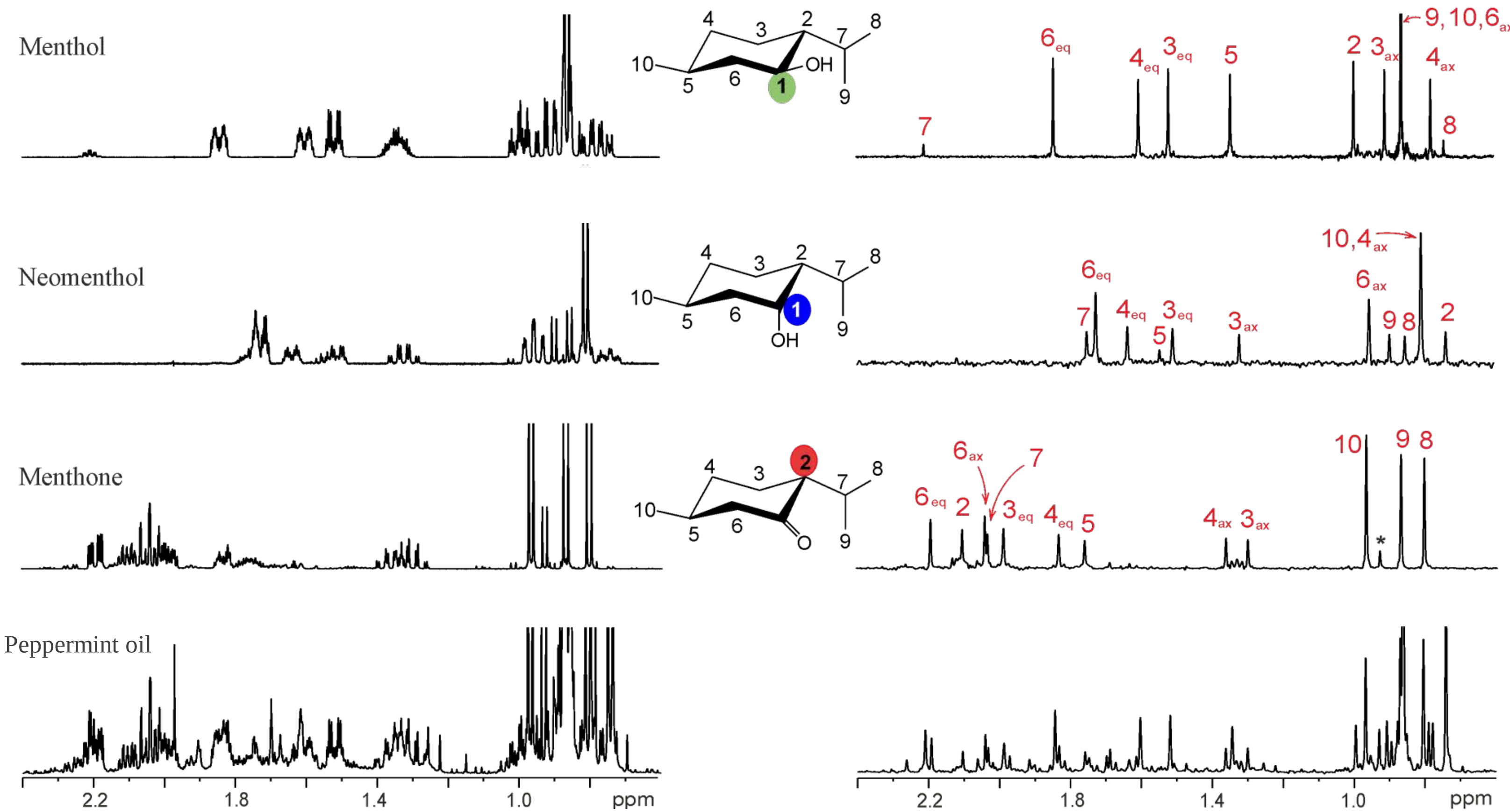
Strychnine

J. Org. Chem. **81**, 11126 (2016)

1D pure shift NMR experiments for the study of complex mixtures

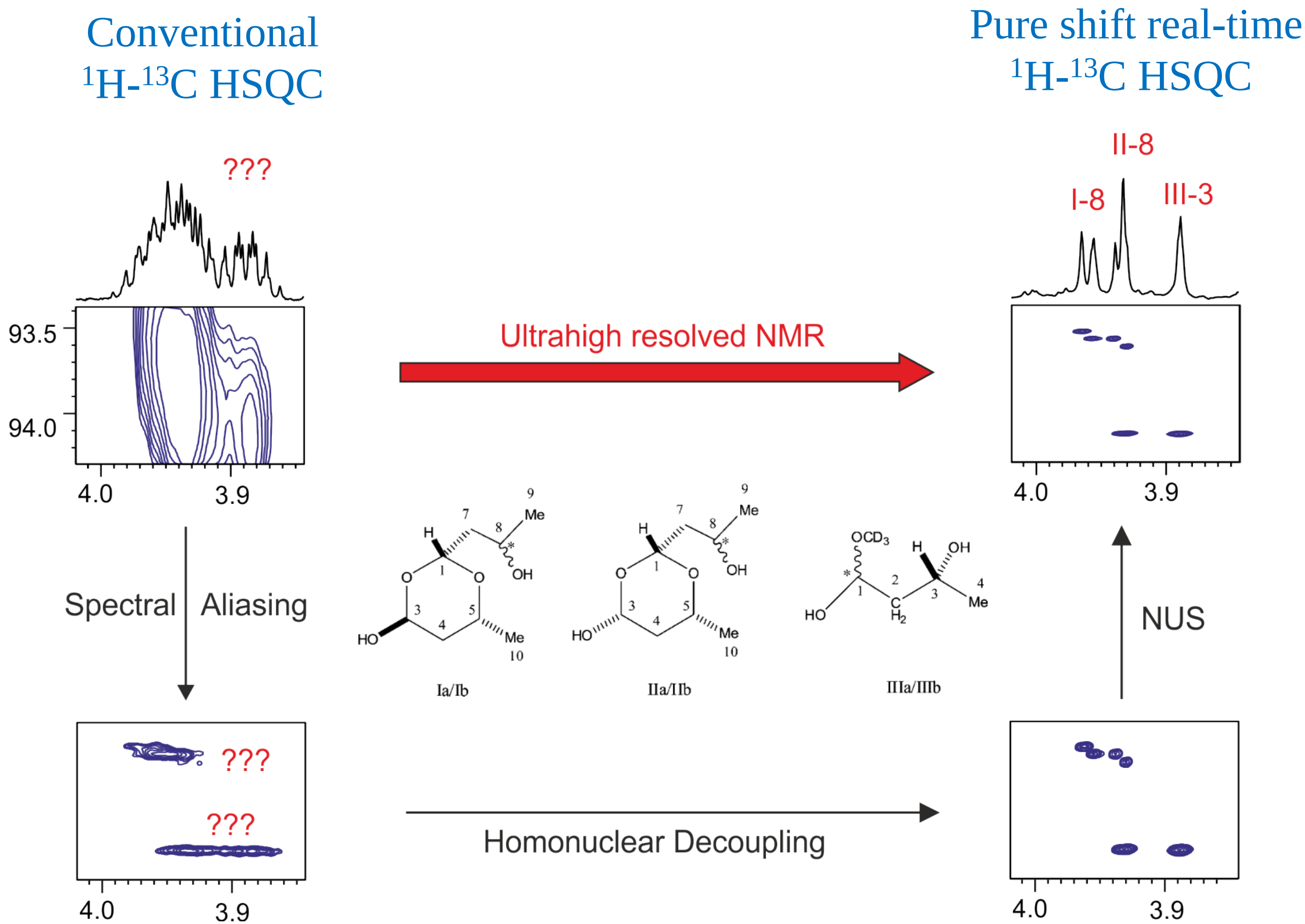
Conventional selective 1D TOCSY

Pure shift interferogram selective 1D PSYCHE-TOCSY



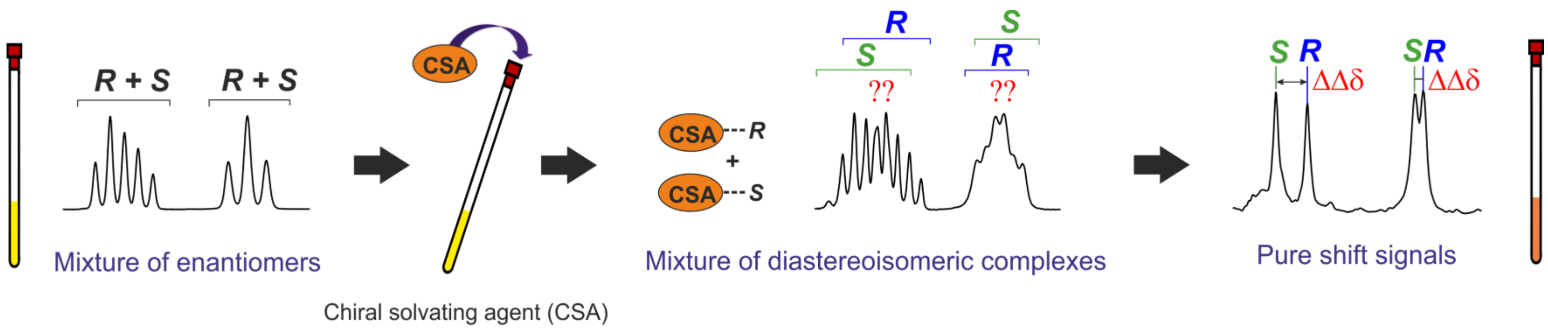
RSC Adv. **6**, 100063 (2016)

2D pure shift NMR experiments for the study of complex mixtures



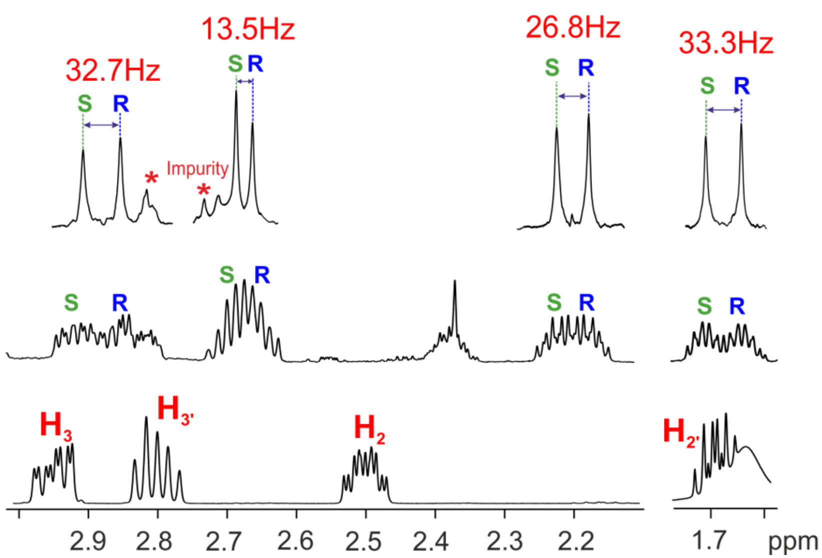
Chem. Eur. J. **21**, 7682 (2015)

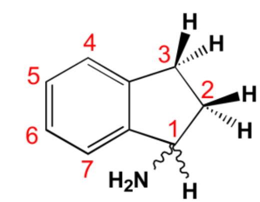
1D pure shift NMR experiments for enantiomer and diastereomer studies



Pure shift real-time BS

Conventional ^{1}H NMR



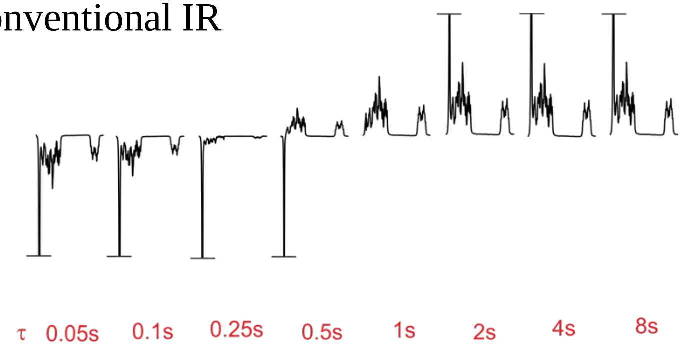


1- aminoindan (racemic mixture)

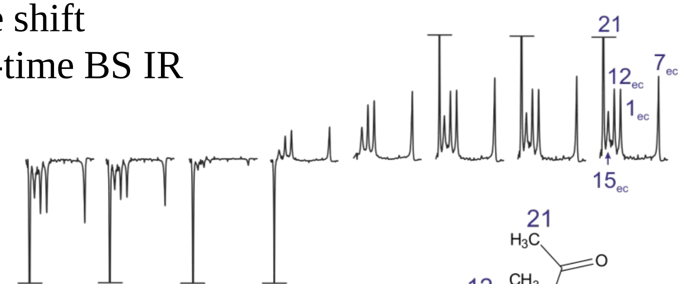
Pure shift NMR experiments to measure relaxation times in overlapped regions

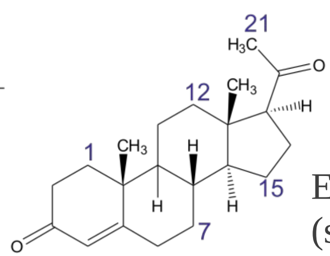
Inversion-Recovery: T_1 measurement

Conventional IR



Pure shift real-time BS IR

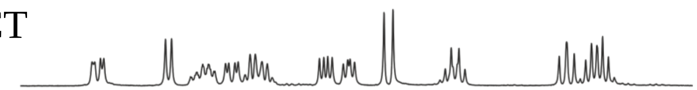




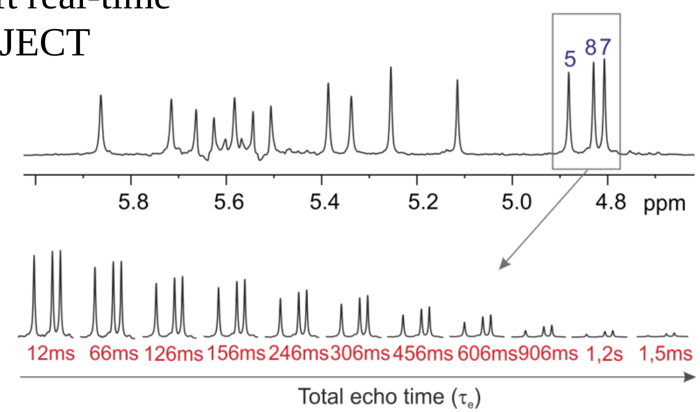
Estradiol (steroid)

PROJECT: Determining T_2 measurement

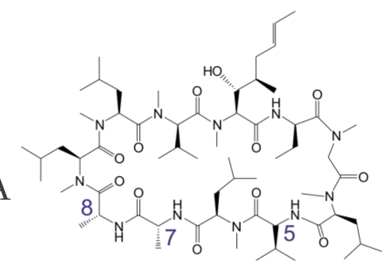
Conventional PROJECT



Pure shift real-time BS PROJECT



Cyclosporine A (peptide)



J. Magn. Reson. **244**, 30 (2014)

Outline

I - Introduction:

Pure shift NMR: setting the scene

Acquisition methods

“Active spin refocusing” methods

Implementation

II - Applications

Structure analysis

Diffusion studies

Measurement of couplings

Mixture analysis

Enantiomeric studies

Dynamic processes

III - Practical aspects:

Sensitivity

Spectral quality

Others

2017 SMASH Workshop Input

1. **Workshop 1: Pure and Simple Understanding Pure Shift NMR Methodology**

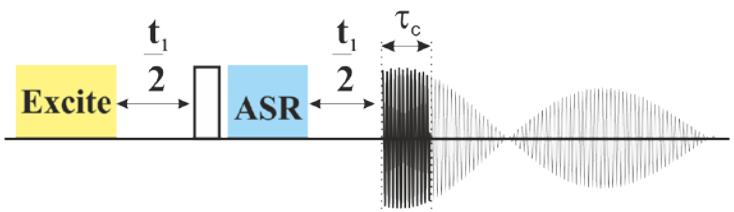
Do you have questions/specific topics you would like this workshop to address?

Q1 – How much S/N do you loose in each method?

General overview: sensitivity of each element

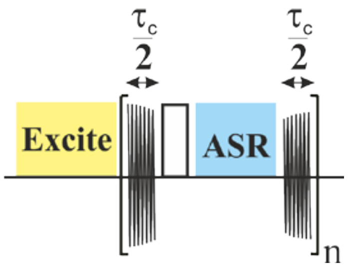
Acquisition mode

Interferogram



$$\propto \frac{1}{\sqrt{number\ of\ increments}}$$

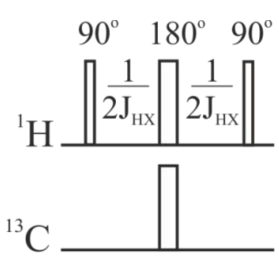
Real-time



100 %

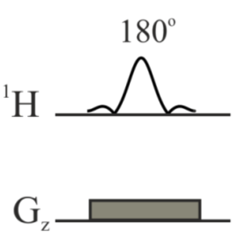
Active spin refocusing methods

BIRD



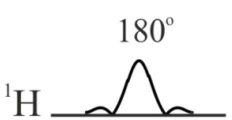
1.1 %

ZS



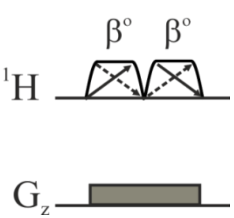
0.5 - 10 %

BS



≥ 100 %

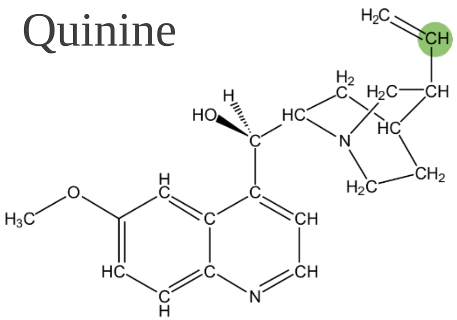
PSYCHE



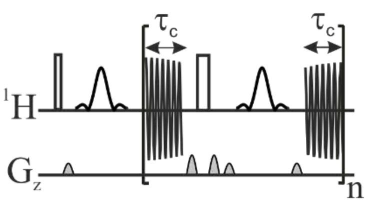
3 - 20 %

Interferogram vs real-time acquisition

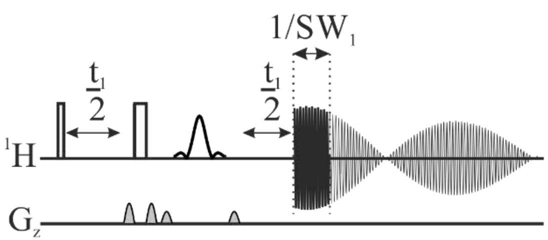
Quinine

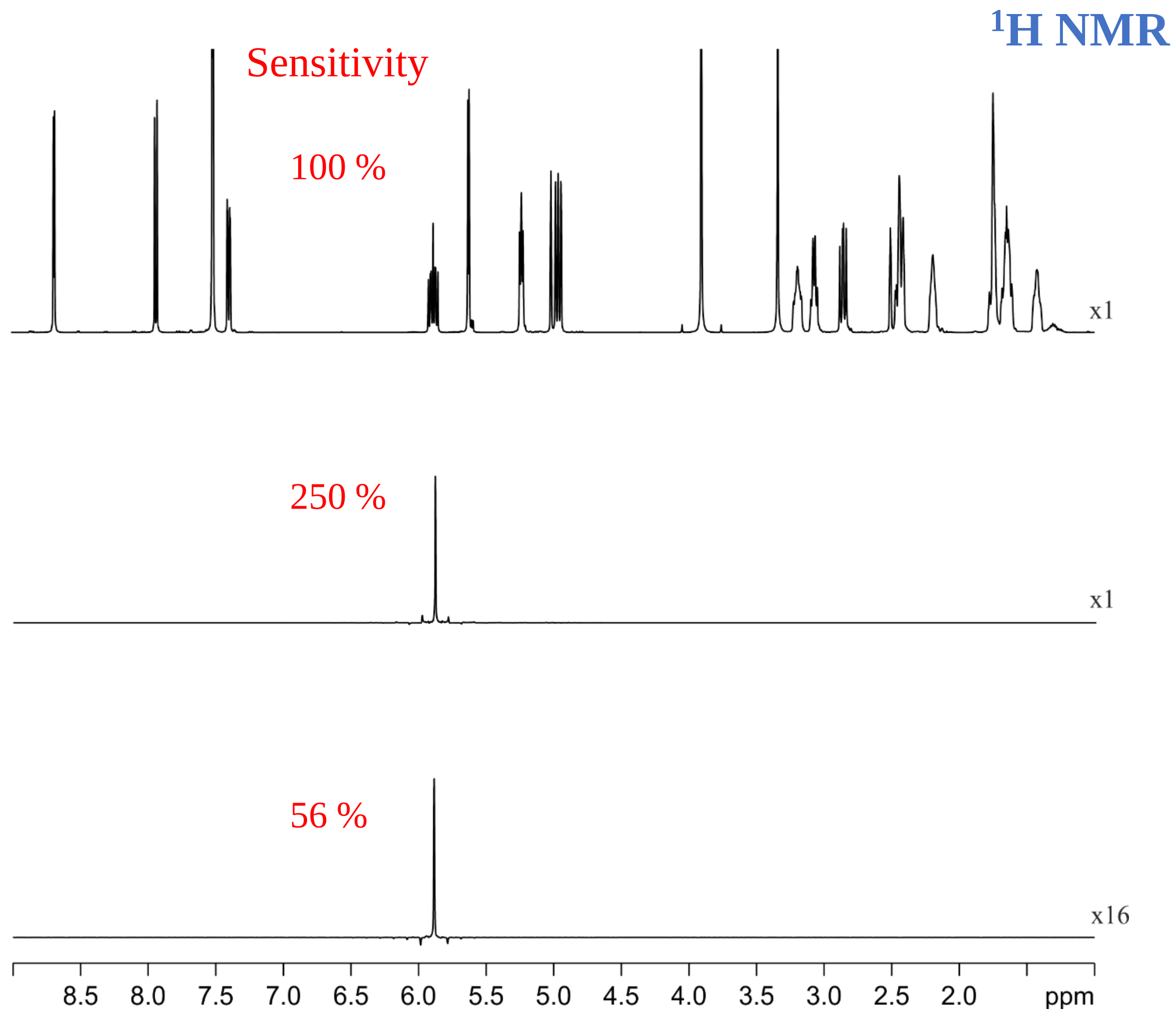


Real-time



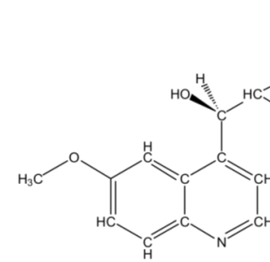
Interferogram





All spectra were acquired with an experiment time of 3 min to compare sensitivity for equal time. Pure shift experiments were acquired using a 20 ms Rsnob selective pulse, and in the interferogram experiment 20 chunks were collected

Comparison of different ASR elements



Quinine

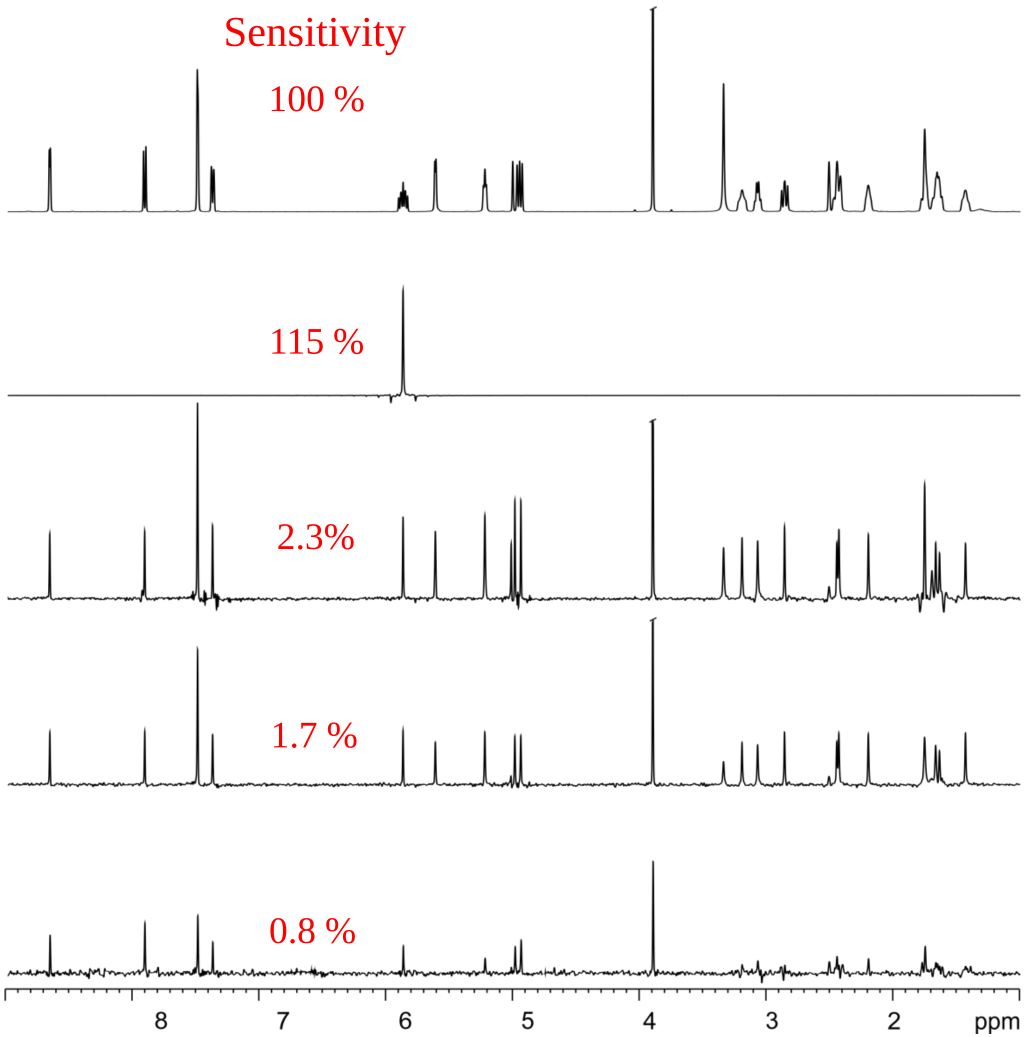
^{1}H NMR

BS

PSYCHE

ZS

BIRD



All spectra were acquired with 4 transients with an experiment time of 52 s (conventional ^{1}H) and 5 min 32 s (pure shift)

Full sensitivity

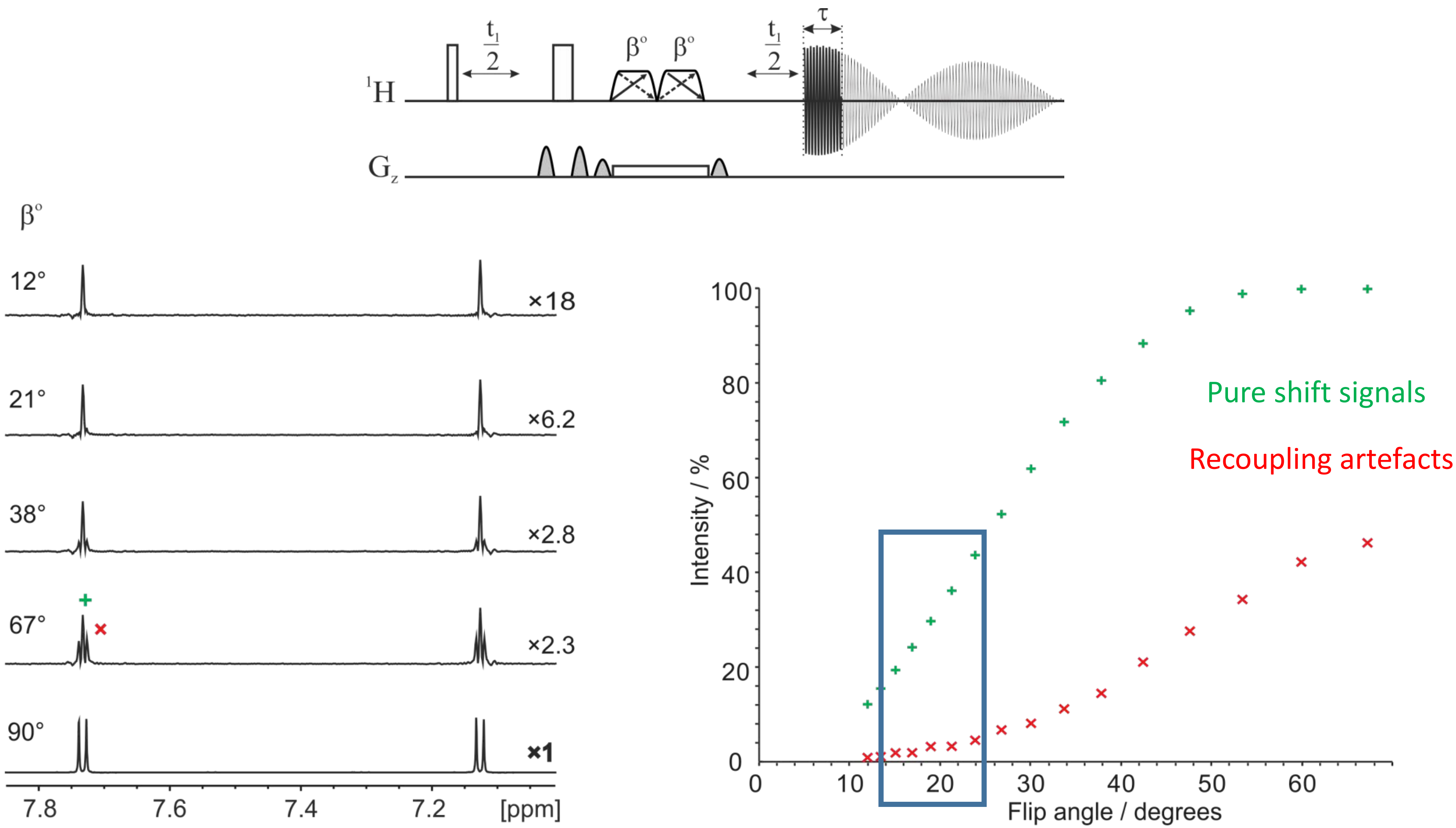
Limited by the β angle of the saltire pulses

Limited by the thickness of the slice

Limited by natural abundance

Real-time 2D HSQC has the same sensitivity as the conventional HSQC

PSYCHE sensitivity limited by β



Adjust the value of β to give the maximum signal-to-noise ratio compatible with the required degree of freedom from spectral artefacts

Angew. Chem. Int. Ed. **53**, 6990 (2014)

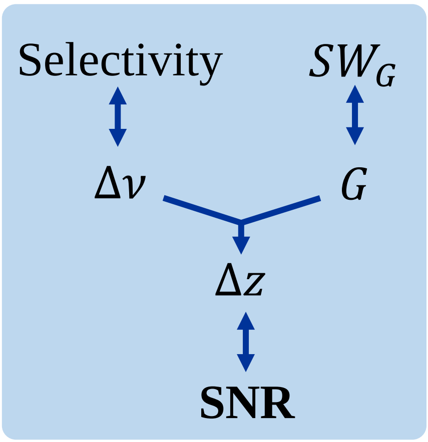
ZS sensitivity limited by the thickness of the slices

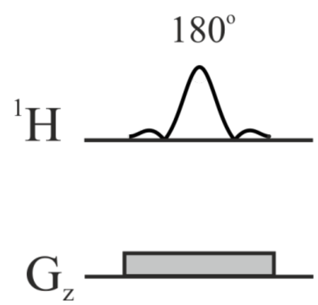
Slice thickness:

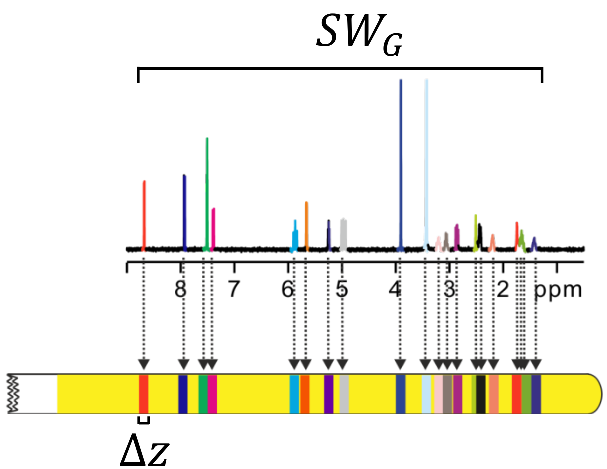
$$\Delta z = \Delta\nu / \frac{\gamma}{2\pi} G$$

Spectral window covered by the gradient:

$$SW_G = \frac{\gamma}{2\pi} L G$$







Typical experimental conditions

- Rsnob pulse ($\Delta\nu$ = 50 Hz)
- SW_G = 5000 Hz
- L = 1.8 cm
- G = 0.65 G/cm

Δz = 0.018 cm

(≈ 100 slices)

Sensitivity ≈ 1%

The selectivity and spectral range required will determine the sensitivity in each case

ZS sensitivity limited by the thickness of the slices

Setting up encoding gradients

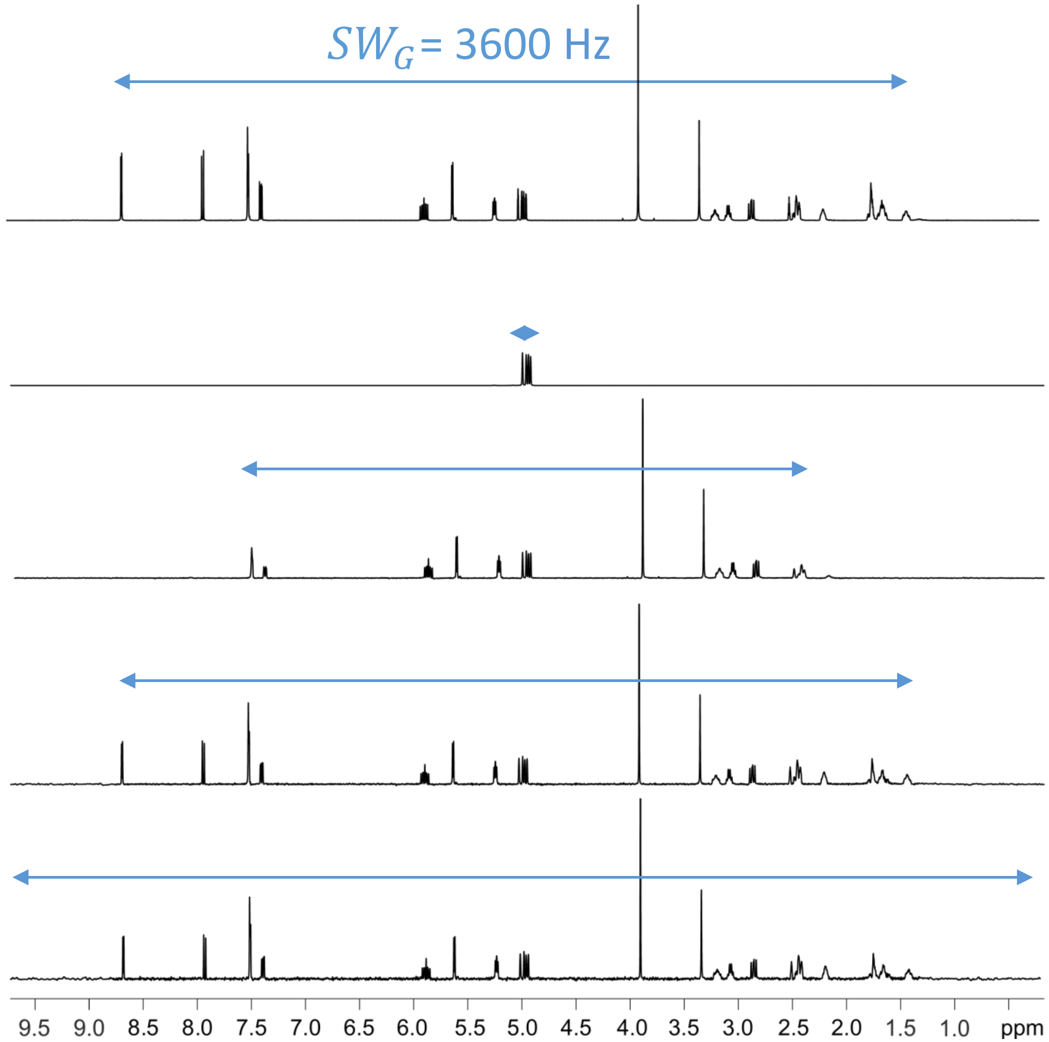
$$G = \frac{2\pi}{\gamma}\frac{SW_G}{L}$$

$G = 0$ G/cm

$G = 0.26$ G/cm (SW_G = 2000 Hz)

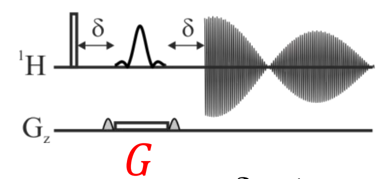
$G = 0.52$ G/cm (SW_G = 4000 Hz)

$G = 1.04$ G/cm (SW_G = 8000 Hz)



^{1}H NMR

Slice selection selective spin echo



δ = 1 ms

ZS sensitivity limited by the thickness of the slices

Setting up encoding gradients

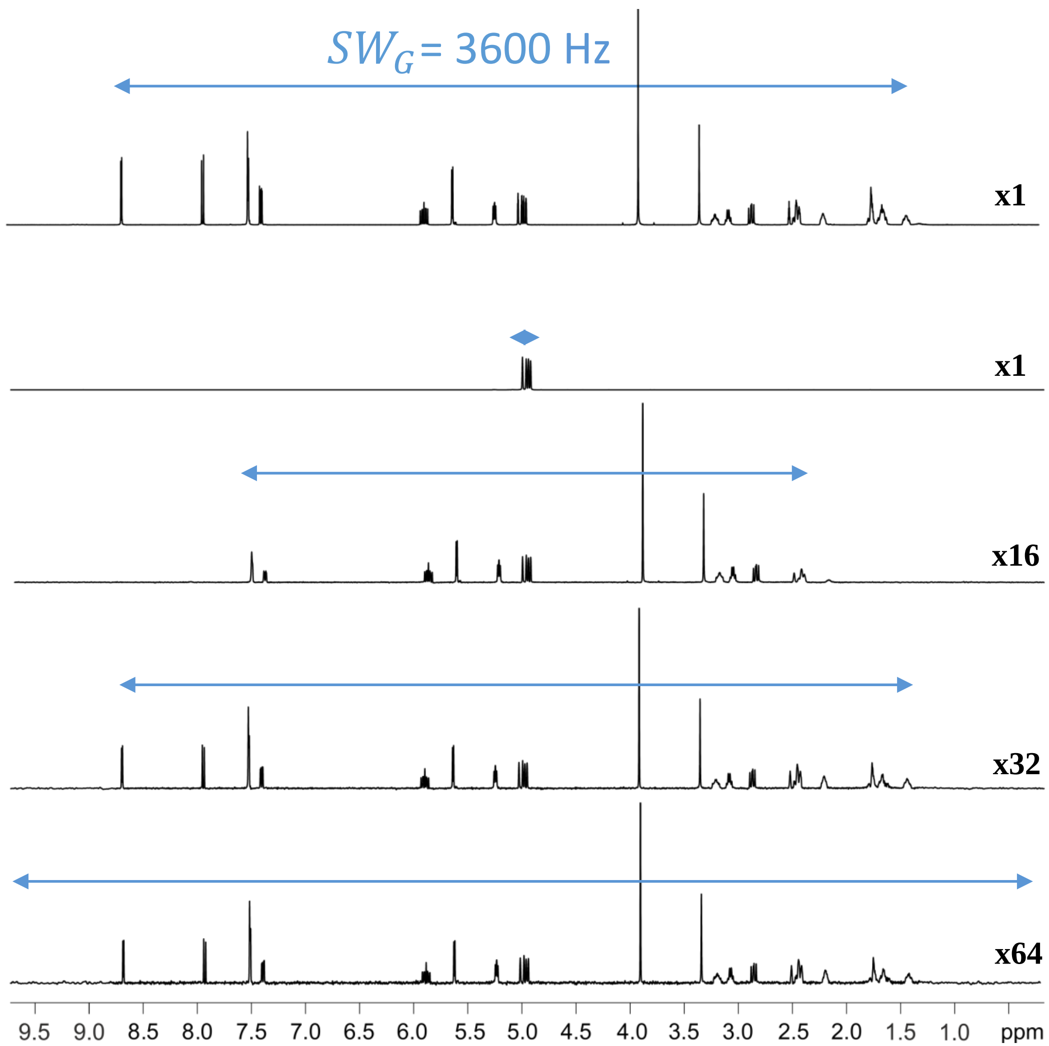
$$G = \frac{2\pi}{\gamma}\frac{SW_G}{L}$$

$G = 0$ G/cm

$G = 0.26$ G/cm (SW_G = 2000 Hz)

$G = 0.52$ G/cm (SW_G = 4000 Hz)

$G = 1.04$ G/cm (SW_G = 8000 Hz)



[1]H NMR

Slice selection selective spin echo

Sensitivity

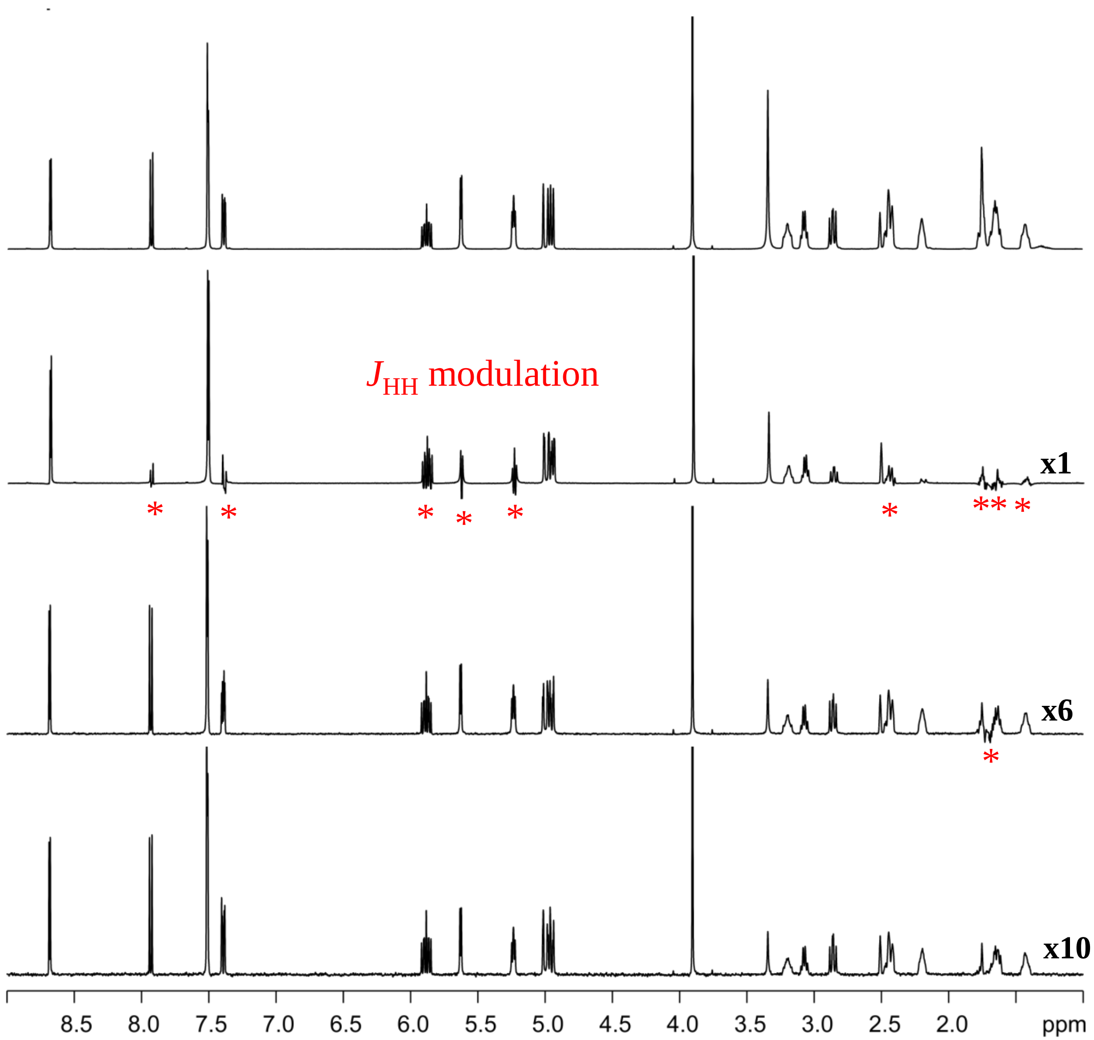
Slice thickness:

$$\Delta z = \Delta\nu / \frac{\gamma}{2\pi} G$$

$\downarrow G \Rightarrow \uparrow \Delta z \Rightarrow \uparrow$ SNR

ZS sensitivity limited by the thickness of the slices

Setting up encoding selective pulse



^{1}H NMR

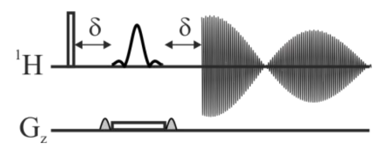
Rsnob

5 ms
($\Delta\nu$ = 466 Hz)

30 ms
($\Delta\nu$ = 78 Hz)

50 ms
($\Delta\nu$ = 47 Hz)

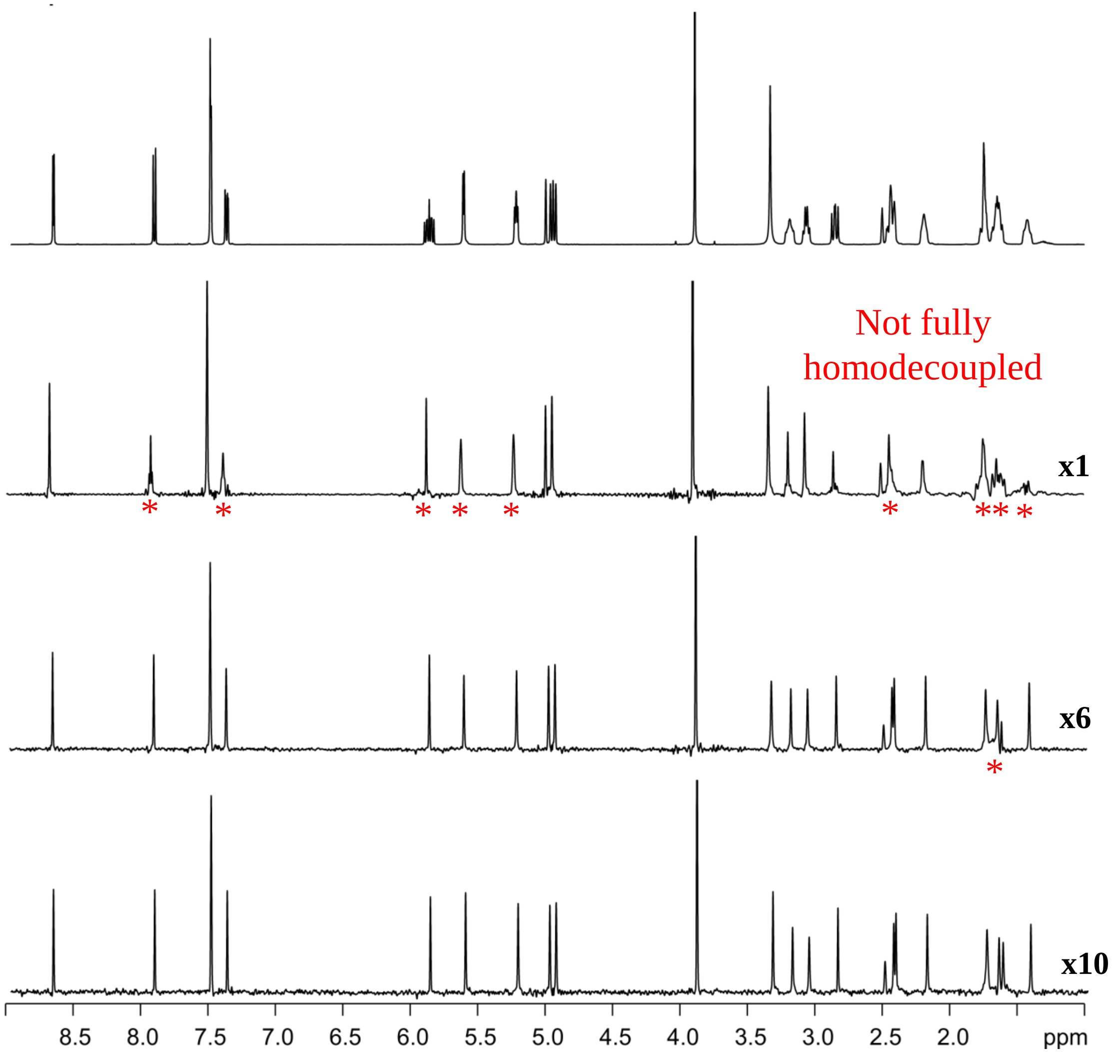
Slice selection selective spin echo



δ = 50 ms
G = 0.52 G/cm

ZS sensitivity limited by the thickness of the slices

Setting up encoding selective pulse



Rsnob

5 ms ($\Delta\nu$ = 466 Hz)

30 ms ($\Delta\nu$ = 78 Hz)

50 ms ($\Delta\nu$ = 47 Hz)

[1]H NMR

ZS interferogram

Sensitivity

Slice thickness:

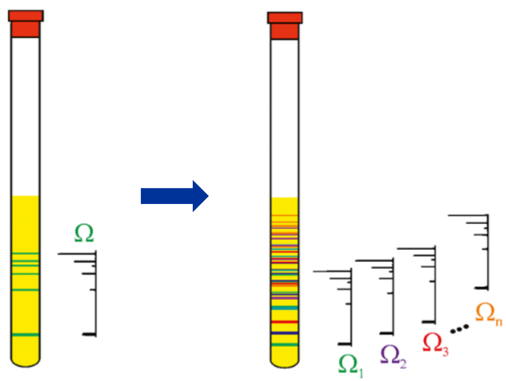
$$\Delta z = \boldsymbol{\Delta\nu} / \frac{\gamma}{2\pi} G$$

$\uparrow \Delta\nu \Rightarrow \uparrow \Delta z \Rightarrow \uparrow$ SNR

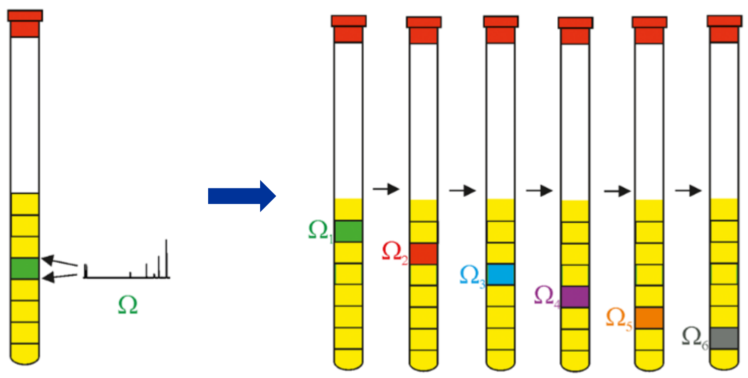
How to increase the sensitivity in ZS experiments?

Using **multiple-frequency selective pulses**

Simultaneous multiple-slice excitation/detection



Sequential spatial excitation and detection



Equidistant *Chem. Eur. J.* **19**, 15472 (2013)

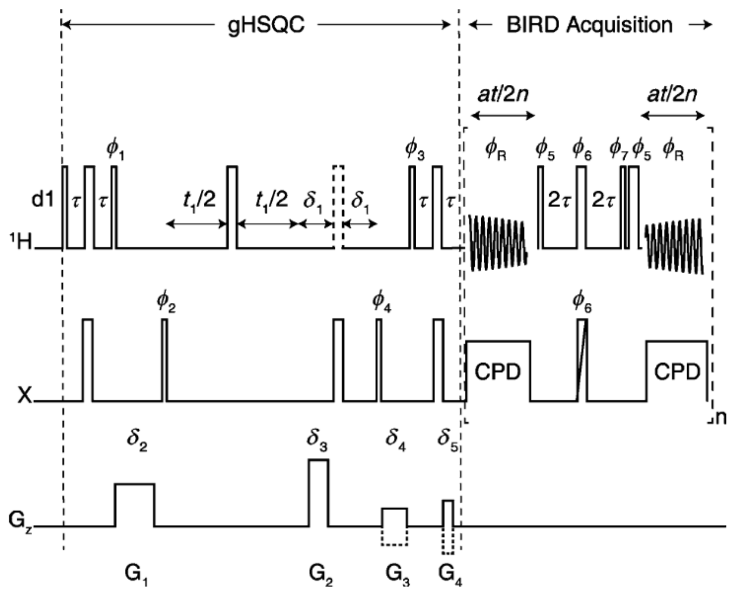
Nonequidistant *Angew. Chem. Int. Ed.* **54**, 6016 (2015)

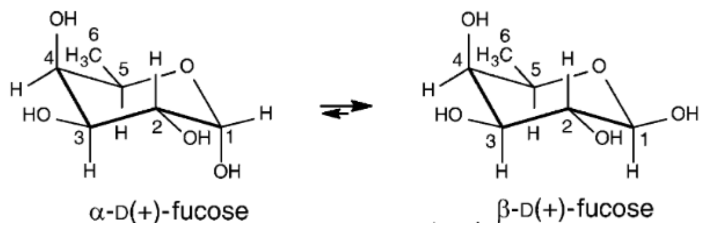
J. Magn. Reson. **233**, 92 (2013)

Using **polarization sharing** to transfer polarization from unutilized protons (passive) to selectively excited (active) protons.

Chem. Commun. **50**, 8550 (2014)

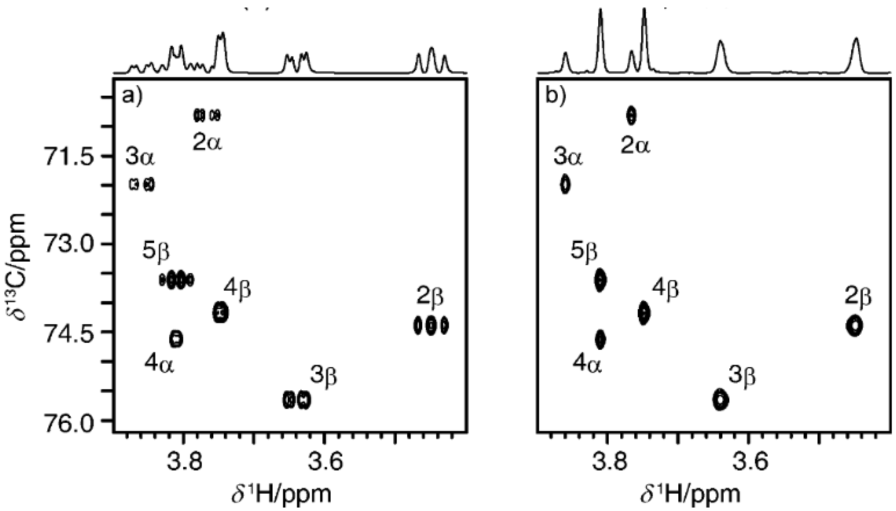
Real-time 2D HSQC – no sensitivity penalty





Conventional ^{1}H-^{13}C HSQC

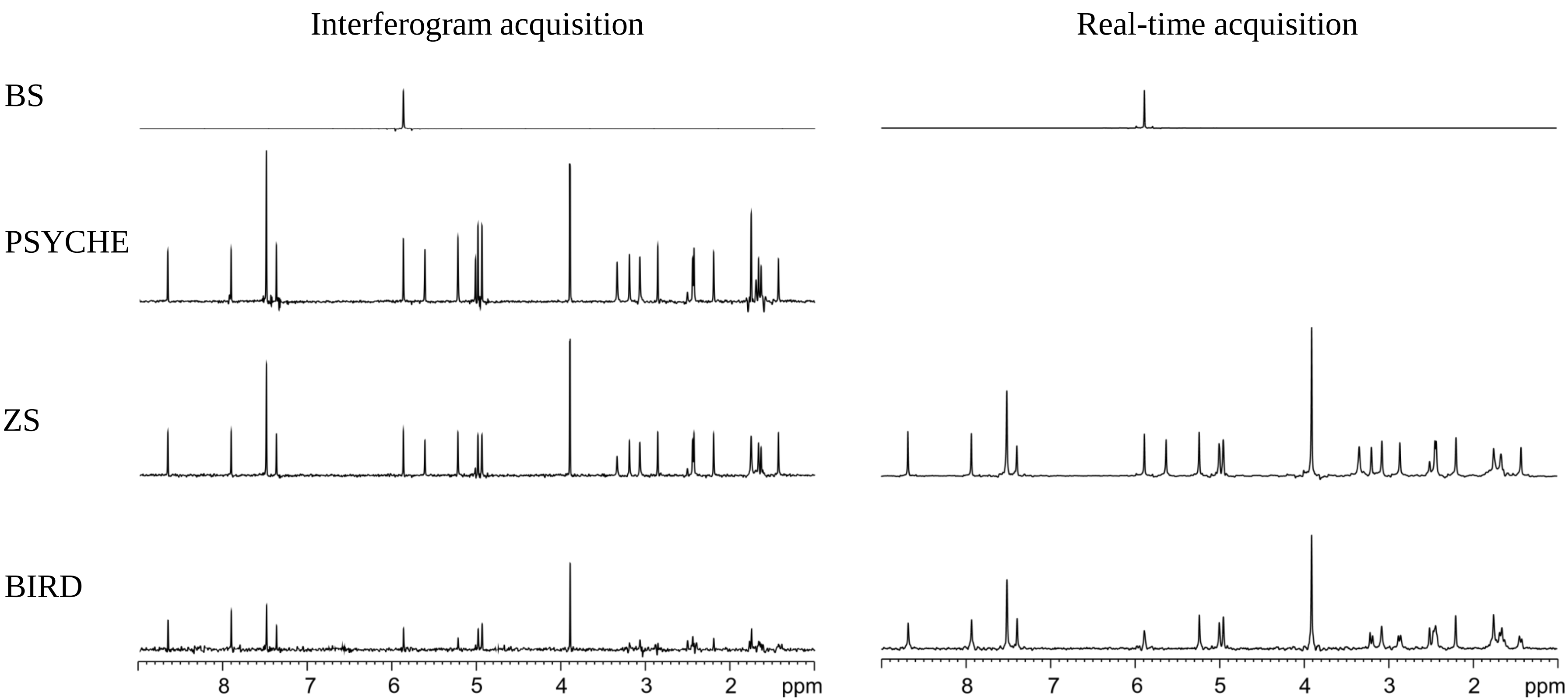
Pure shift real-time ^{1}H-^{13}C HSQC



Simultaneous enhancement of spectral resolution and sensitivity

Angew. Chem. Int. Ed. **52**, 11616 (2013)

What should we consider when talking about spectral quality?



Resolution
Digital resolution
Line broadening

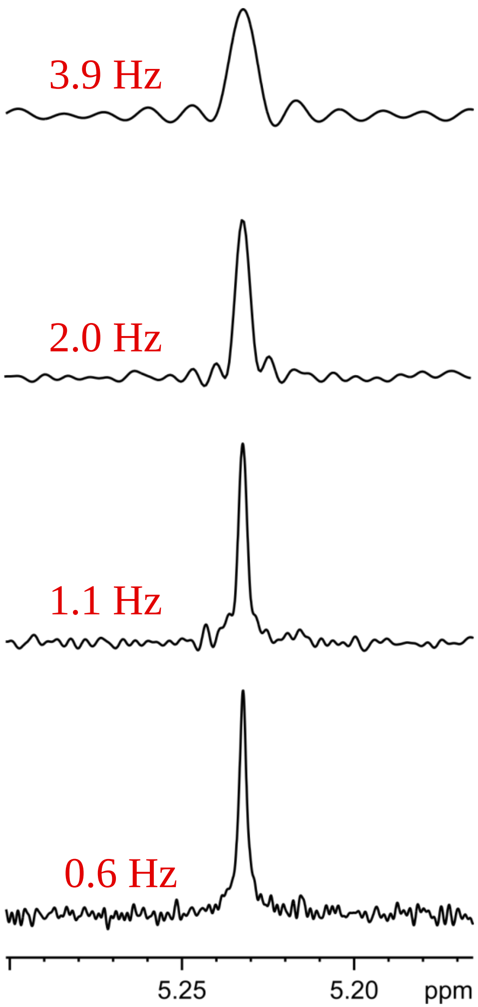
Artefacts
Chunking sidebands
Digital filter artefacts
Phase discontinuity artefacts
Fast pulsing artefacts

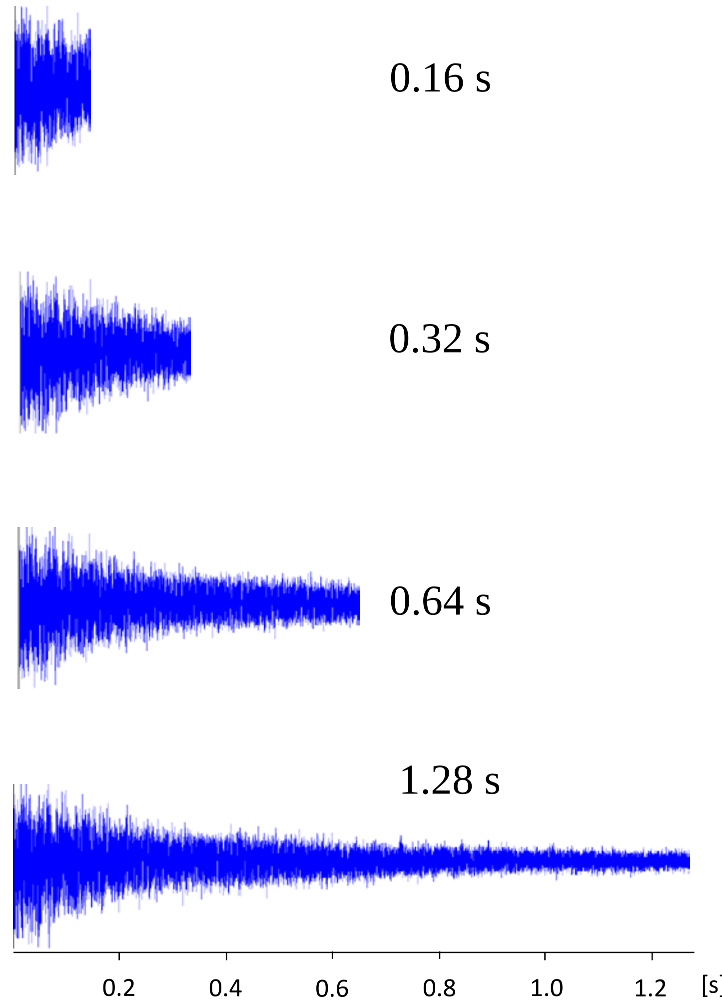
Unwanted signals
Recoupling signals
Strong coupling signals

Effects of pulse miscalibration and B_1 inhomogeneity

Signal resolution in interferogram pure shift NMR experiments

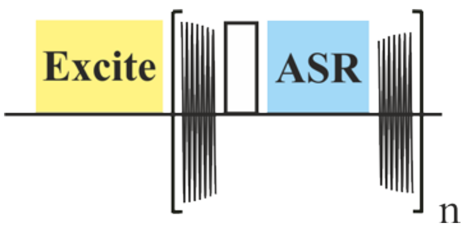
line-width at half height	number of chunks	number of FID points	duration of the complete interferogram (pure shift acquisition time)
3.9 Hz	8	3200	0.16 s
2.0 Hz	16	6400	0.32 s
1.1 Hz	32	12800	0.64 s
0.6 Hz	64	25600	1.28 s





Signal resolution in real-time pure shift NMR experiments

Signal resolution in real-time experiments depends on:



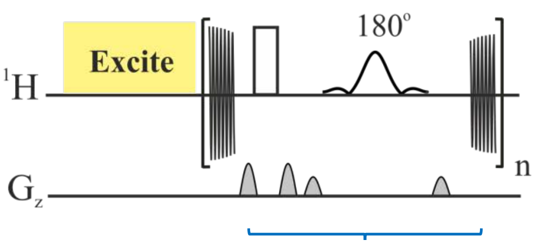
FID resolution

Increasing the number of chunks improves digital resolution…
… but loses more signal and in real-time experiments more problems with irreproducibility from chunk to chunk occurs

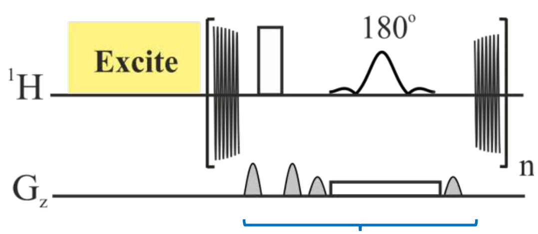
Duration of the *J*-refocusing element

Increasing *J*-refocusing time loses more signal by T_2 relaxation.
This makes the FID to decay faster (broader signals)…
…and also generates discontinuity from chunk to chunk (bigger chunking artefacts)

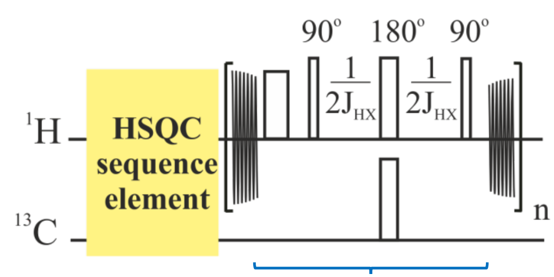
BS



ZS



BIRD



The duration on the *J*-refocusing element depends on the duration of the selective pulse (selectivity requirements)

< 10 ms

$^1J_{CH}$ = 120 – 200 Hz

$1/(2\,^1J_{CH})$ = 4 – 2.5 ms

Signal resolution in BS and ZS real-time pure shift NMR experiments

Rsnob

BS

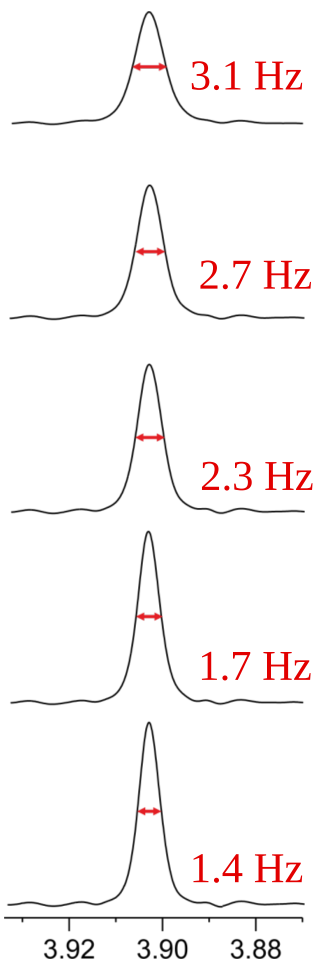
50 ms
($\Delta\nu$ = 47 Hz)

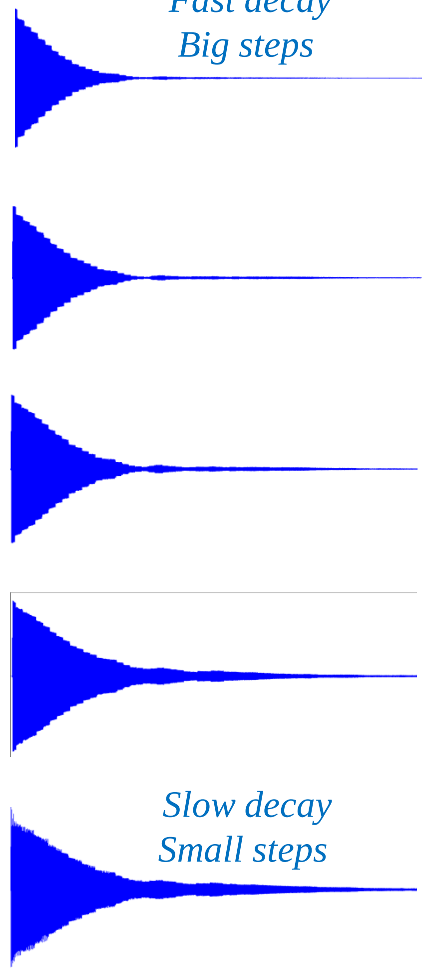
30 ms
($\Delta\nu$ = 78 Hz)

20 ms
($\Delta\nu$ = 117 Hz)

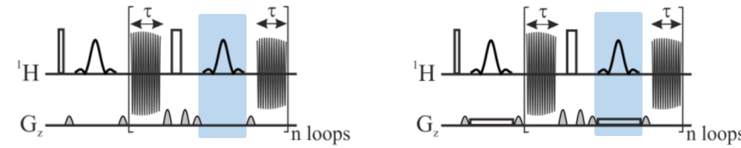
10 ms
($\Delta\nu$ = 233 Hz)

5 ms
($\Delta\nu$ = 466 Hz)





BS **ZS**



Increase the selectivity

⬇

Increase the duration

⬇

Longer gap between chunks

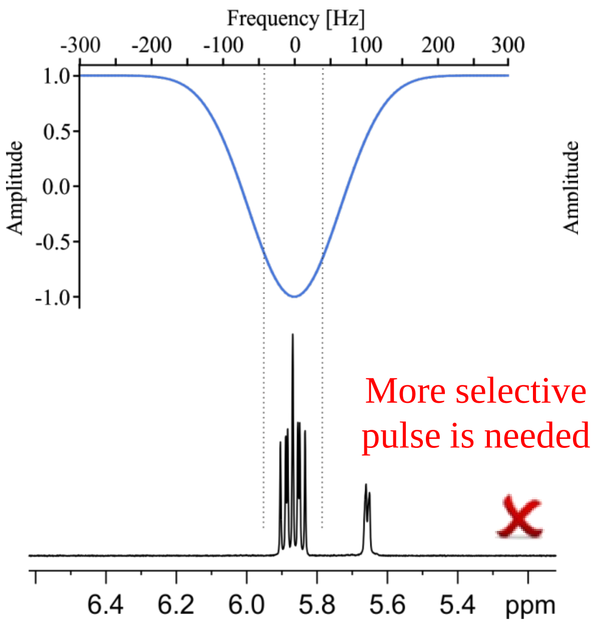
⬇

Bigger step in the FID

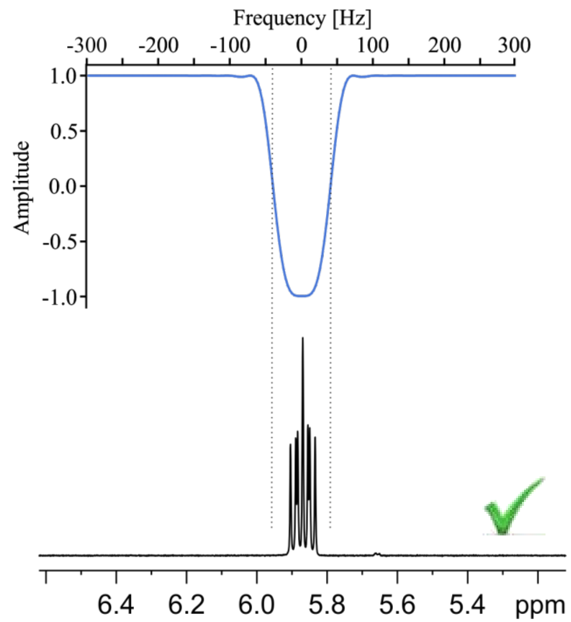
- Faster FID decay ⬇ Greater linewidth
- Bigger chunking artefacts

Selective pulses: shape and duration

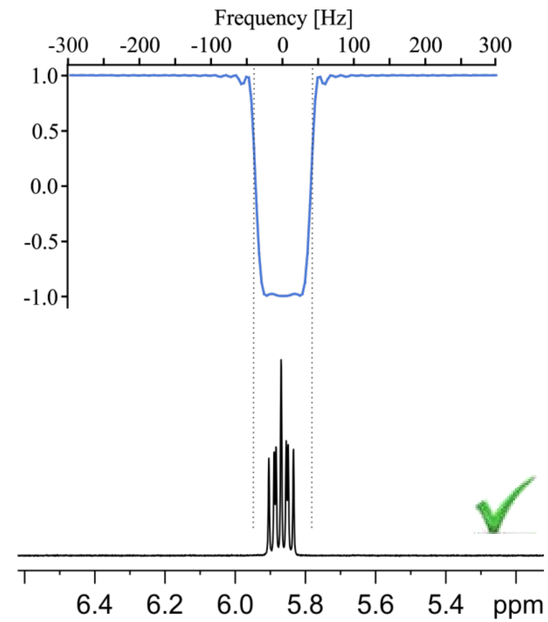
Gauss
$\Delta\nu$ = 88 Hz
Duration = 10 ms



Rsnob
$\Delta\nu$ = 88 Hz
Duration = 30 ms

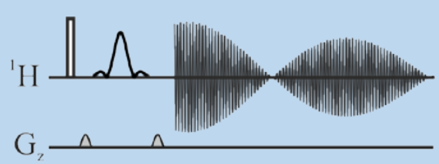


ReBurp
$\Delta\nu$ = 88 Hz
Duration = 70 ms



Very selective pulses can be used to deal with strong coupling

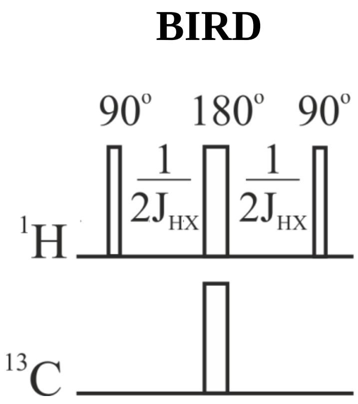
Pulse sequence used: selective spin echo



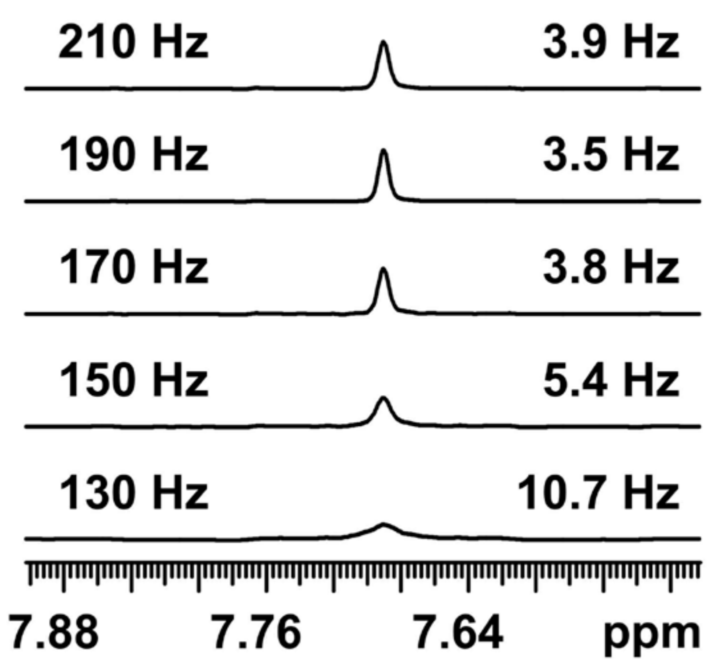
Bruker pp: selgpse

Signal resolution in BS and ZS real-time pure shift NMR experiments

Effect of BIRD timing error

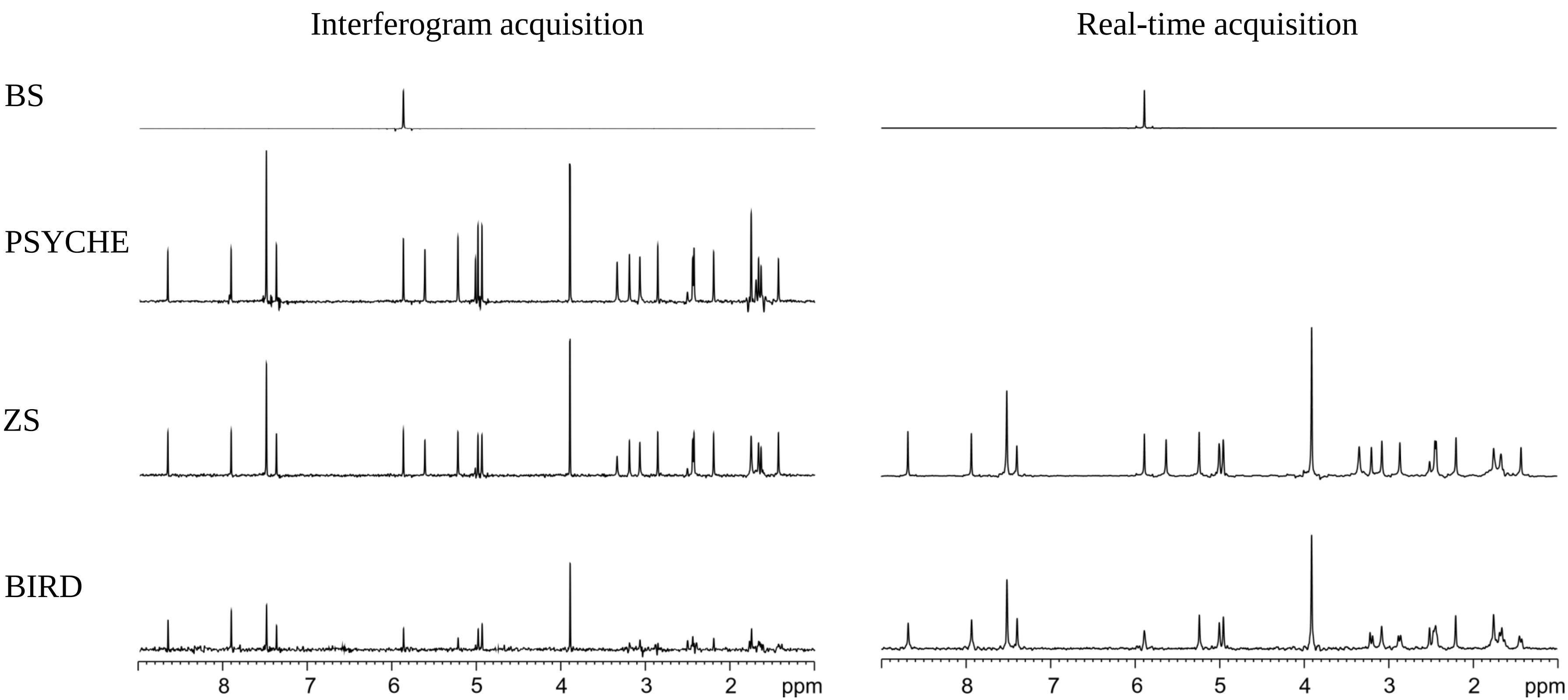


Timing errors in the BIRD element lead to signal broadening



Selected traces of real-time pure shift BIRD-HSQC ($^{1}J_{CH}$ = 190 Hz). The echo time ($1/^{1}J_{CH}$) of the BIRD element was varied (HSQC sequence element was kept constant)

What should we consider when talking about spectral quality?



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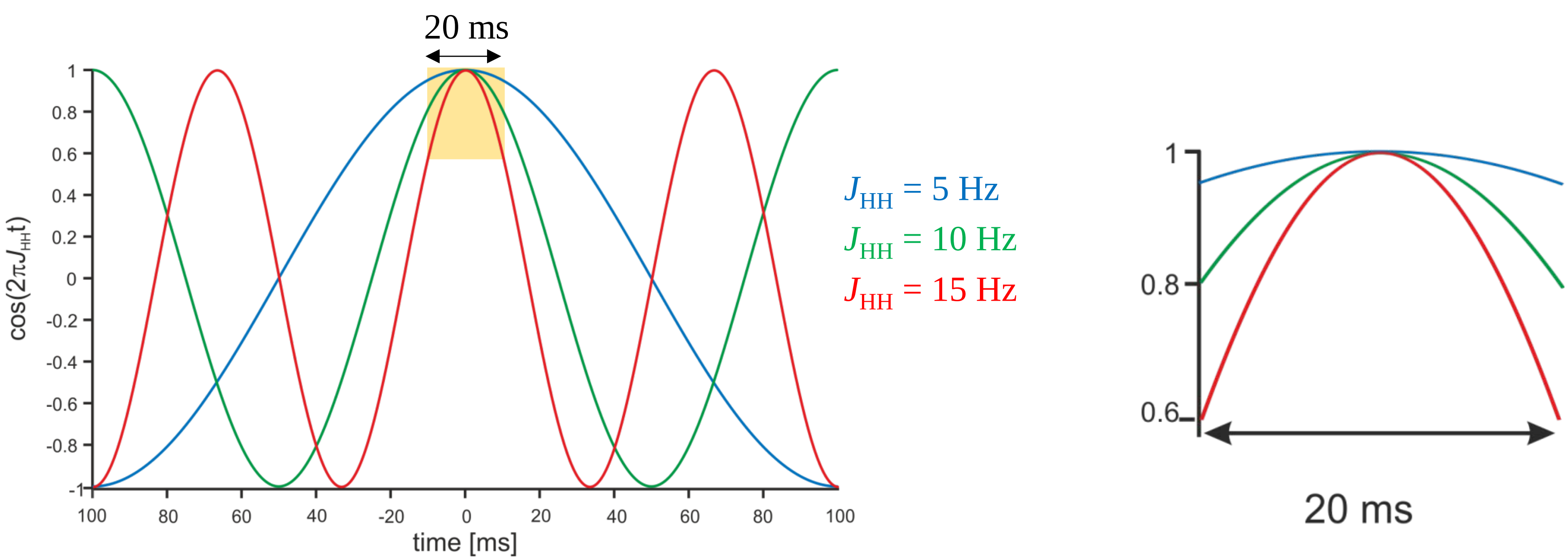
Chunking sidebands: the origin

$J << \delta$ allows data acquisition in ‘chunks’ lasting $<< 1/J_{HH}$ (J-evolution during the chunk is negligible)



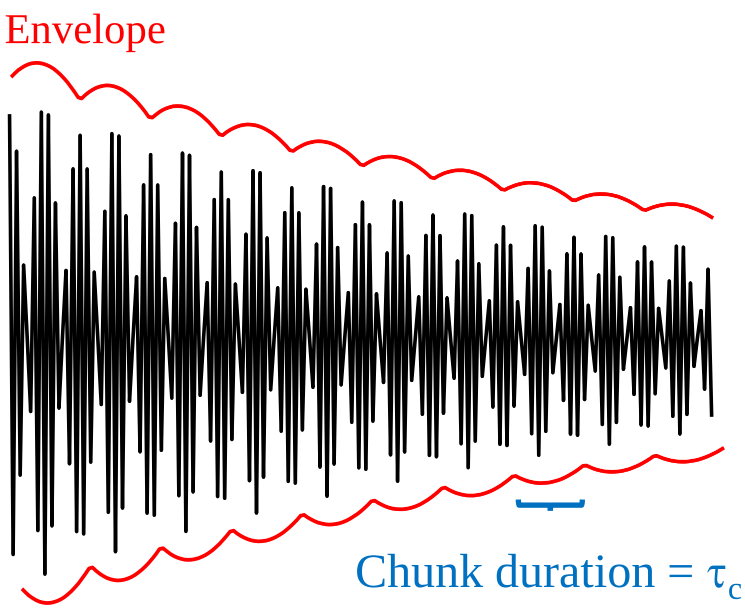
Typically pure shift experiments are acquire with a chunk duration (τ_c) ≈ 20-40 ms

Interferogram acquisition: $\tau_c = 1/SW_1$
Real-time acquisition: $\tau_c = AQ/n$

Chunking sidebands: effect on the pure shift NMR spectrum

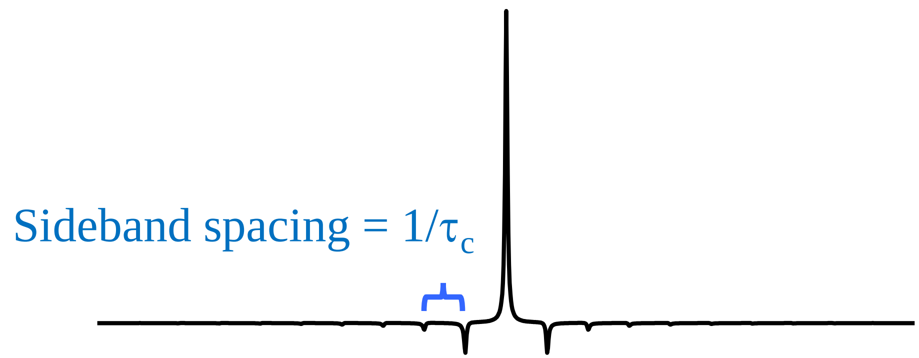
Acquiring pure shift data in chunks of duration τ_c gives rise to *J*-sidebands with a spacing $1/\tau_c$ in the spectrum

Pure shift FID



interferogram: $\tau_c = 1/SW_1$
real-time: $\tau_c = AQ/n$

Pure shift spectrum



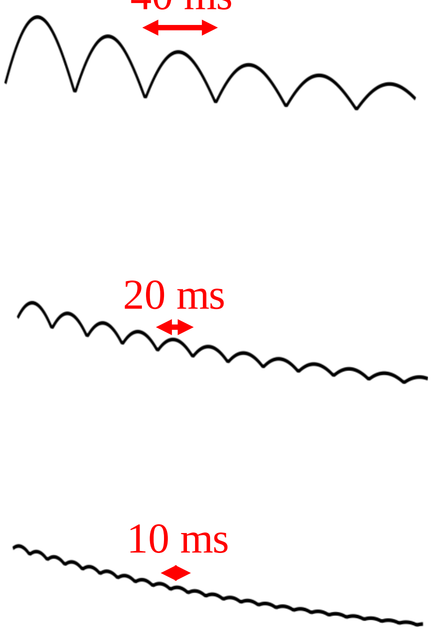
Amplitude of the n^{th} sideband for an AX system:

$$(\cos^2[\pi n] - \alpha n \cot[\pi / 2\alpha] \sin[2\pi n]) / (1 - 4\alpha n^2)$$

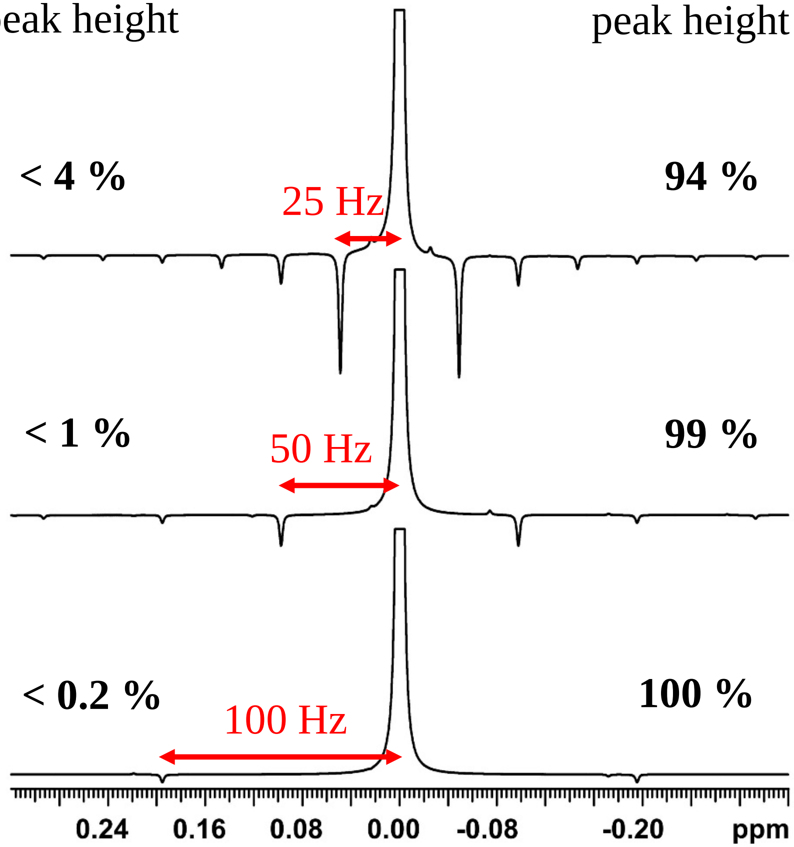
where $\alpha = 1 / (J_{HH}\, \tau_c)$

Chunking sidebands: effect of chunk duration (τ)

Pure shift FID envelope



Pure shift spectrum



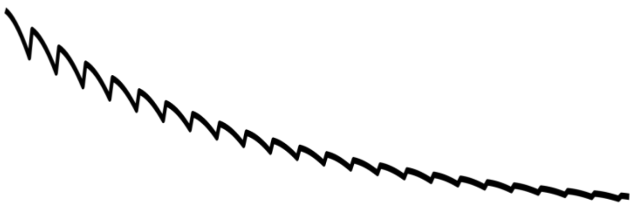
side-bands peak height	pure shift peak height	duration of chunk τ_c ($1/\tau_c$)	number of chunks
< 4 %	**94 %**	40 ms (25 Hz)	32
< 1 %	**99 %**	20 ms (50 Hz)	64
< 0.2 %	**100 %**	10 ms (100 Hz)	128

Interferogram band-selective pure shift spectra of an AX spin system calculated in Matlab/Spinach. (AQ = 1.3 s; J_{AX} = 10 Hz)

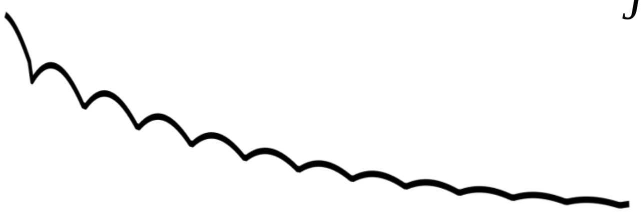
Chunking sidebands: effect of *J* refocusing position

Pure shift FID envelope

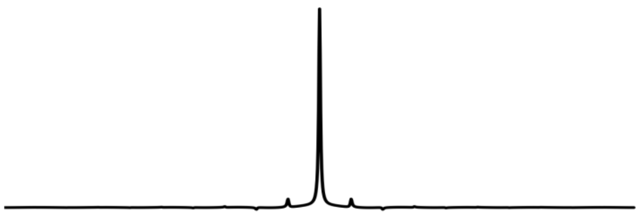
Pure shift spectrum

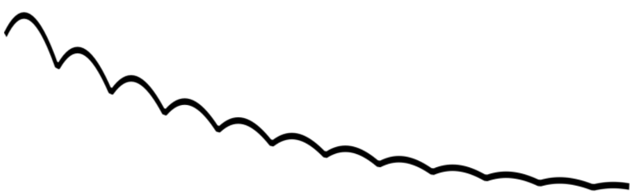


J refocused at the beginning of each chunk

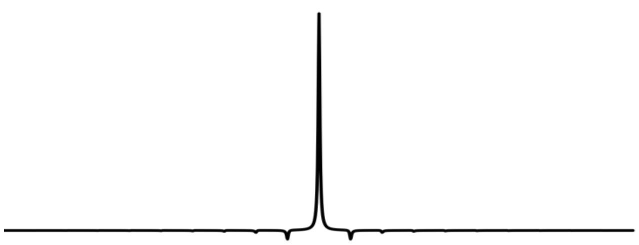


J refocused at the beginning of the first (half) chunk and in the middle of successive ones





J refocused in the middle of each chunk



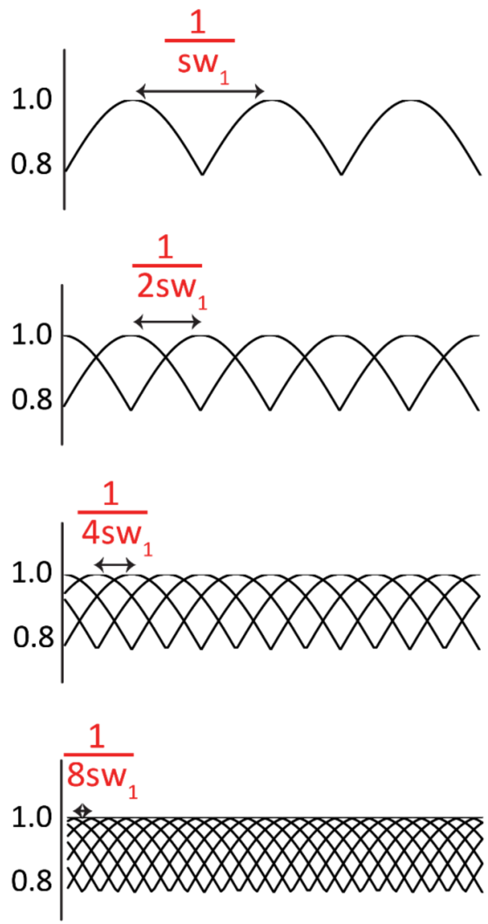
How to suppress chunking sidebands?

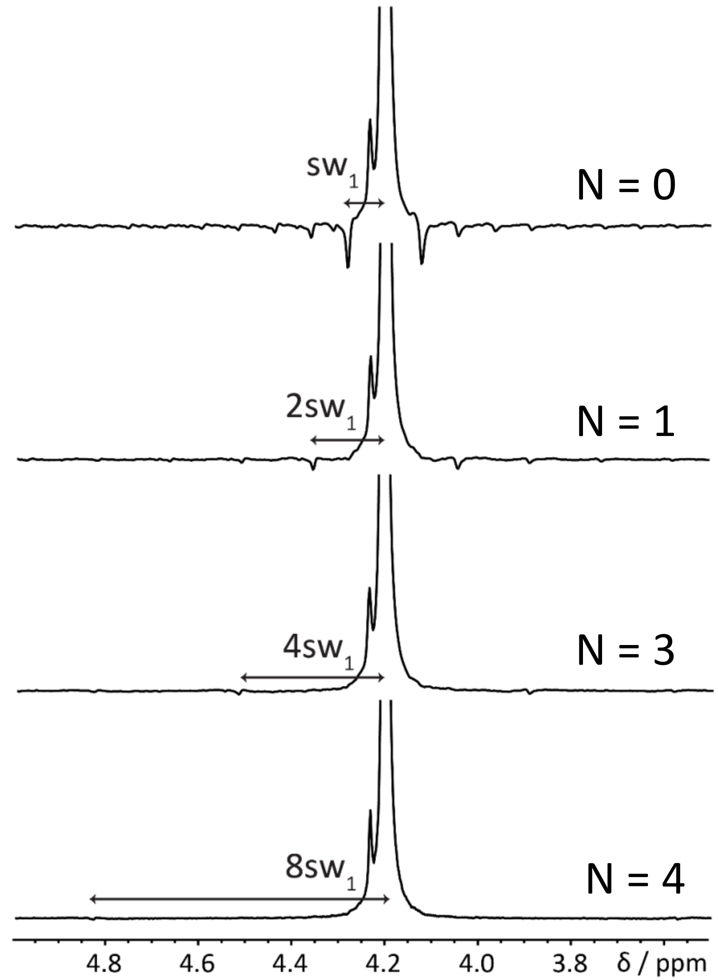
SAPPHIRE: getting rid of sidebands by modulation averaging

Systematically varying the timing of the first chunk suppresses sidebands to order N in N+1 experiments

Averaging spectra measured with different τ can reduce sidebands, but does not suppress them

J. Magn. Reson. **259**, 207 (2015)



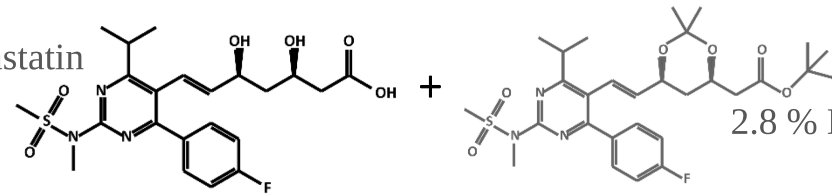


Chem. Commun. **53**, 10188 (2017)

How to suppress chunking sidebands?

SAPPHIRE: getting rid of sidebands by modulation averaging

97.2 % rosuvastatin

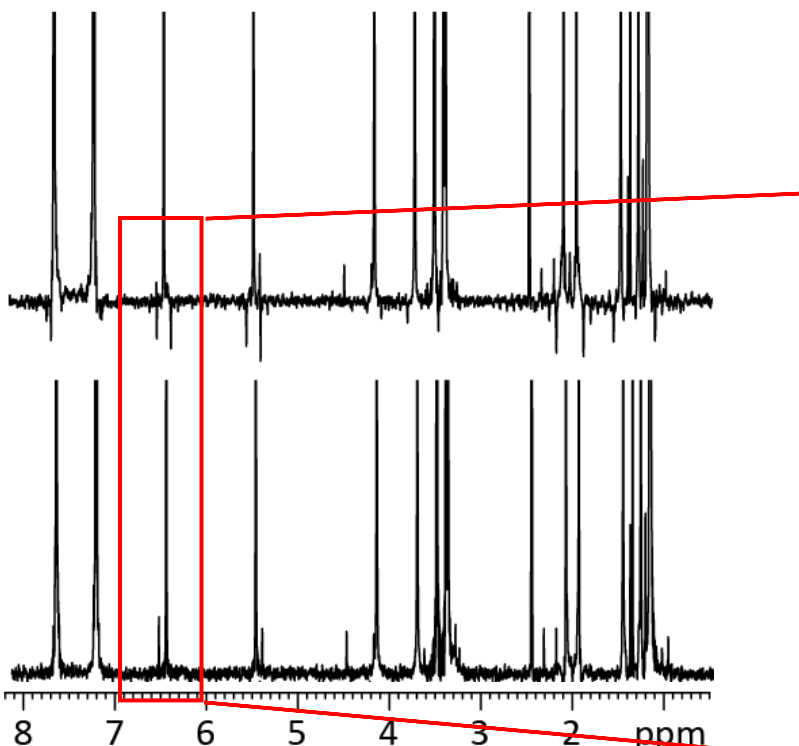


2.8 % BEM (precursor)

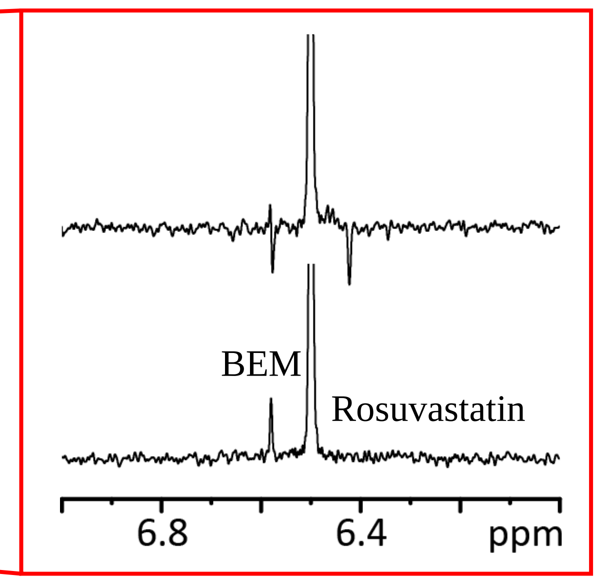
POSTER 10

Conventional interferogram ZS pure shift spectrum

Interferogram SAPPHIRE ZS pure shift spectrum



A signal of BEM is accidentally cancelled by overlap with a negative sideband.

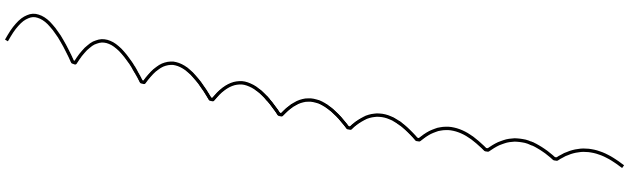


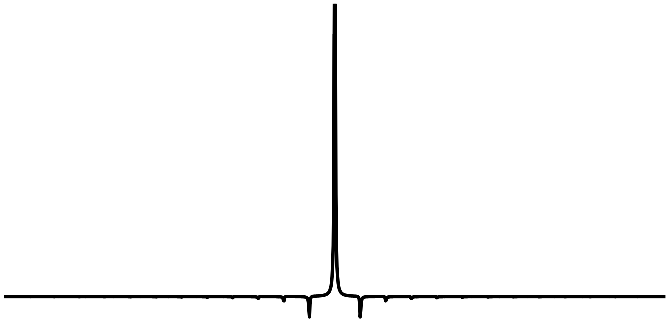
Effect of distorted early data points

In practice the first few data points of a FID are always distorted
Modern digital filtering methods often make this worse

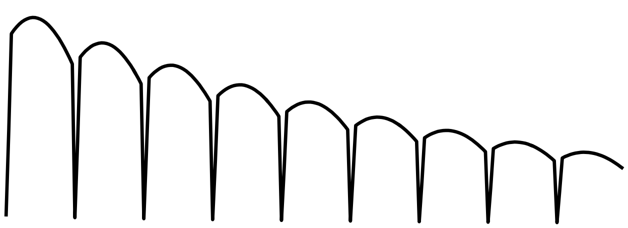
FID envelope | **Pure shift spectrum**

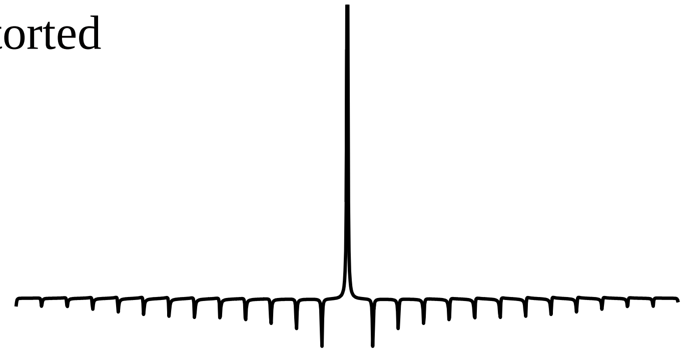
No distortion





First two points distorted





Interferogram acquisition: effect of $SW/SW_1 \neq$ integer

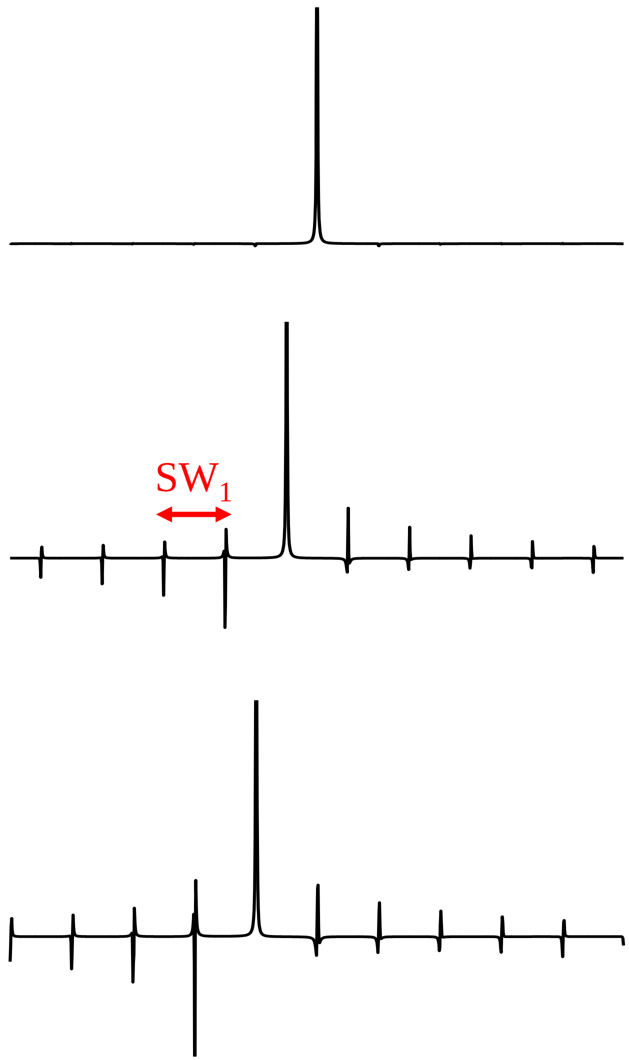
SW/SW_1 = **9.8**

(SW_1 = 102 Hz, SW = 1000 Hz)

δ = 0 Hz (on resonance)
Only weak *J*-sidebands

δ = 50 Hz

δ = 100 Hz



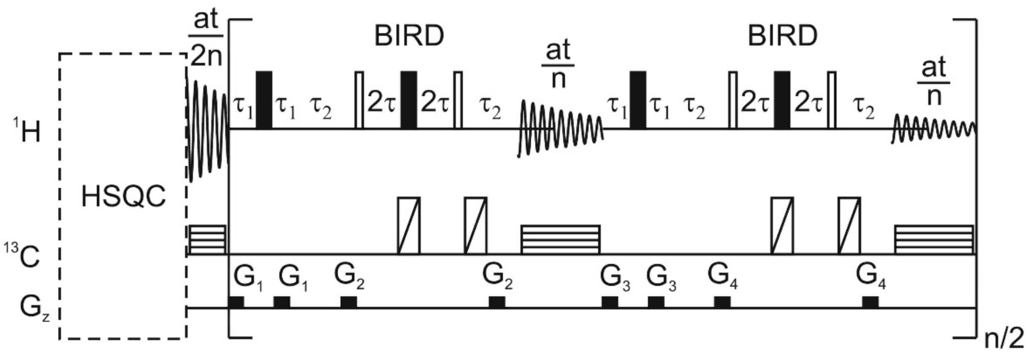
Frequency-dependent phase discontinuity between chunks

Real-time 2D HSQC

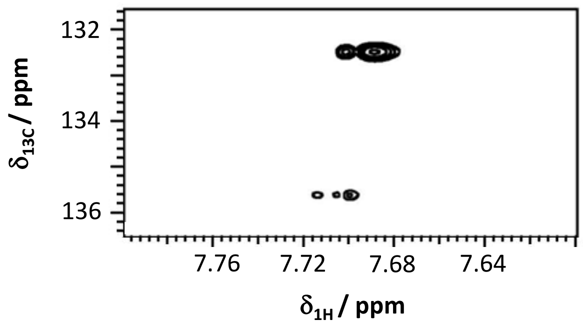
Pure shift

real-time

^{1}H-^{13}C gHSQC

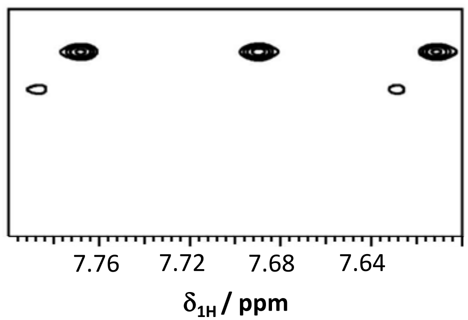


Gradient pulses omitted,
basic phase cycle,
and no supercycle…



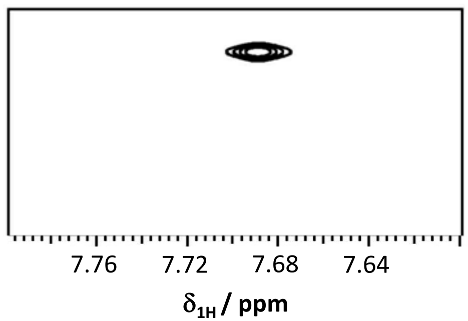
… extra signals next to the pure shift signal and F_1 mirror images

2^{nd} carbon 180 pulse is omitted in BIRD…



… extra signals appear due to heteronuclear *J*-evolution during τ_2

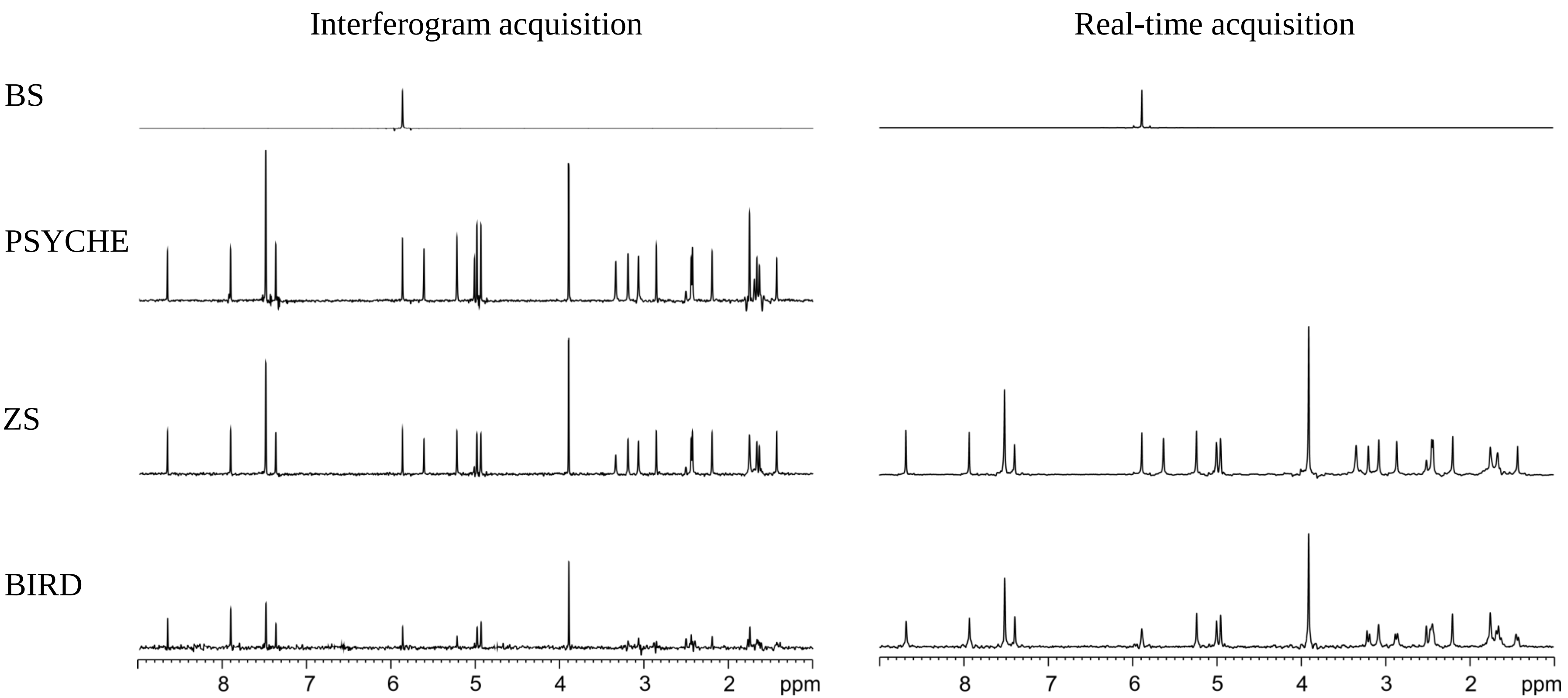
$G_{1\text{-}4}$ varied chunk-to-chunk
extended phase cycle (*2),
and MLEV-16 supercycle…



… clean

J. Biomol. NMR **62**, 43 (2015)

What should we consider when talking about spectral quality?



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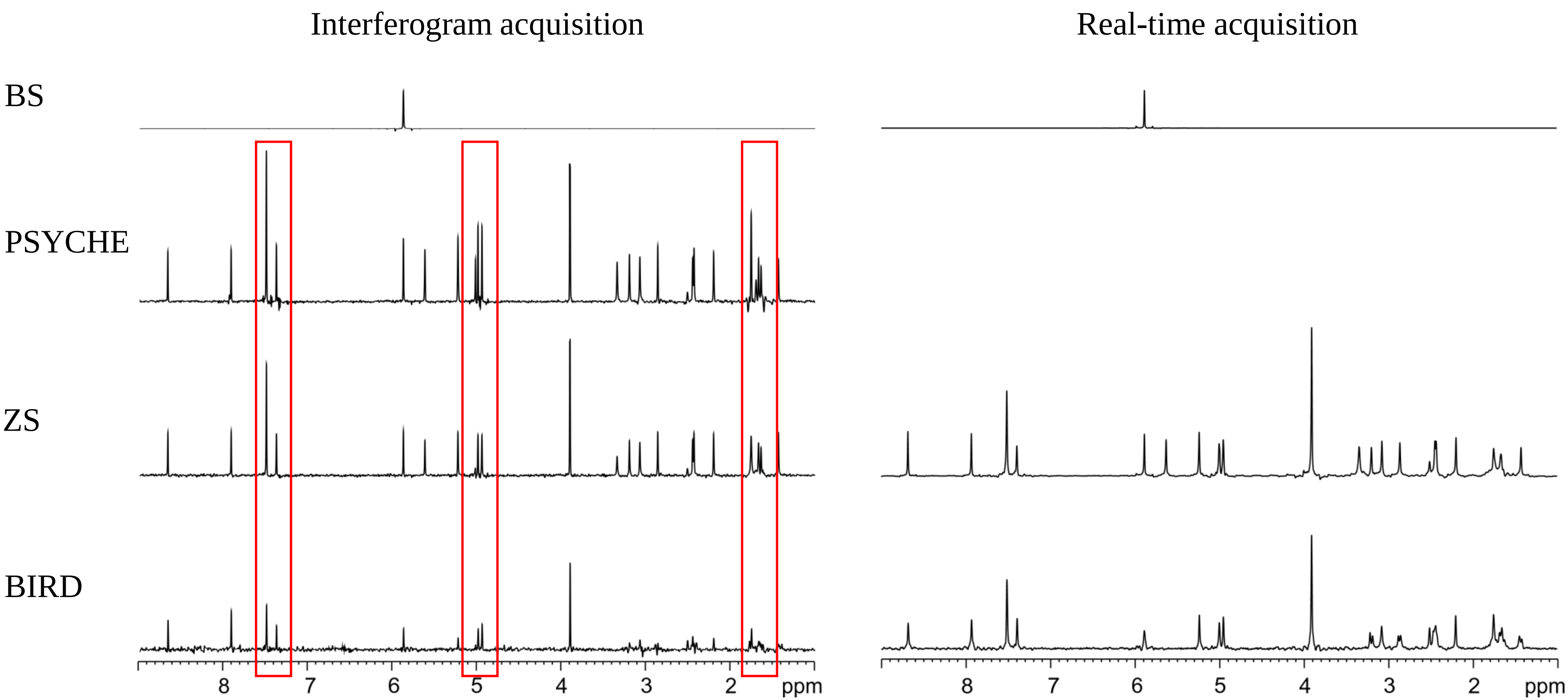

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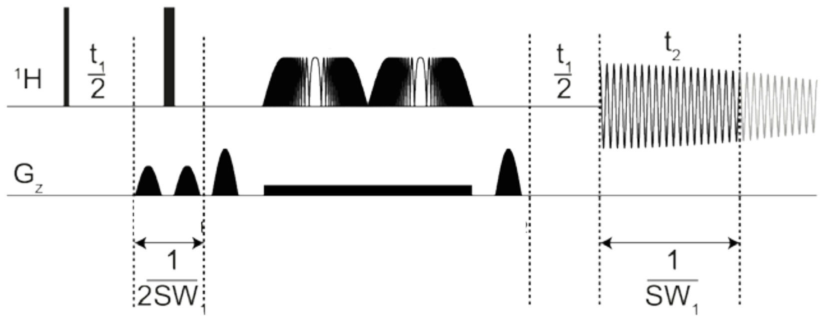
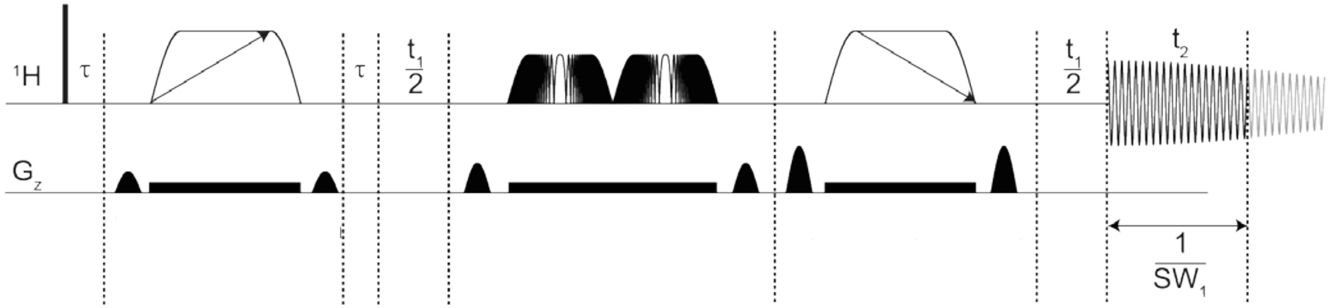
Strong coupling: PSYCHE vs TSE-PSYCHE

In triple spin echo (TSE) PSYCHE the addition of two extra 180° chirp pulses in the presence of weak pulsed field gradients instead of a hard 180° pulse results in additional spatiotemporal averaging and significant improvement in spectral quality

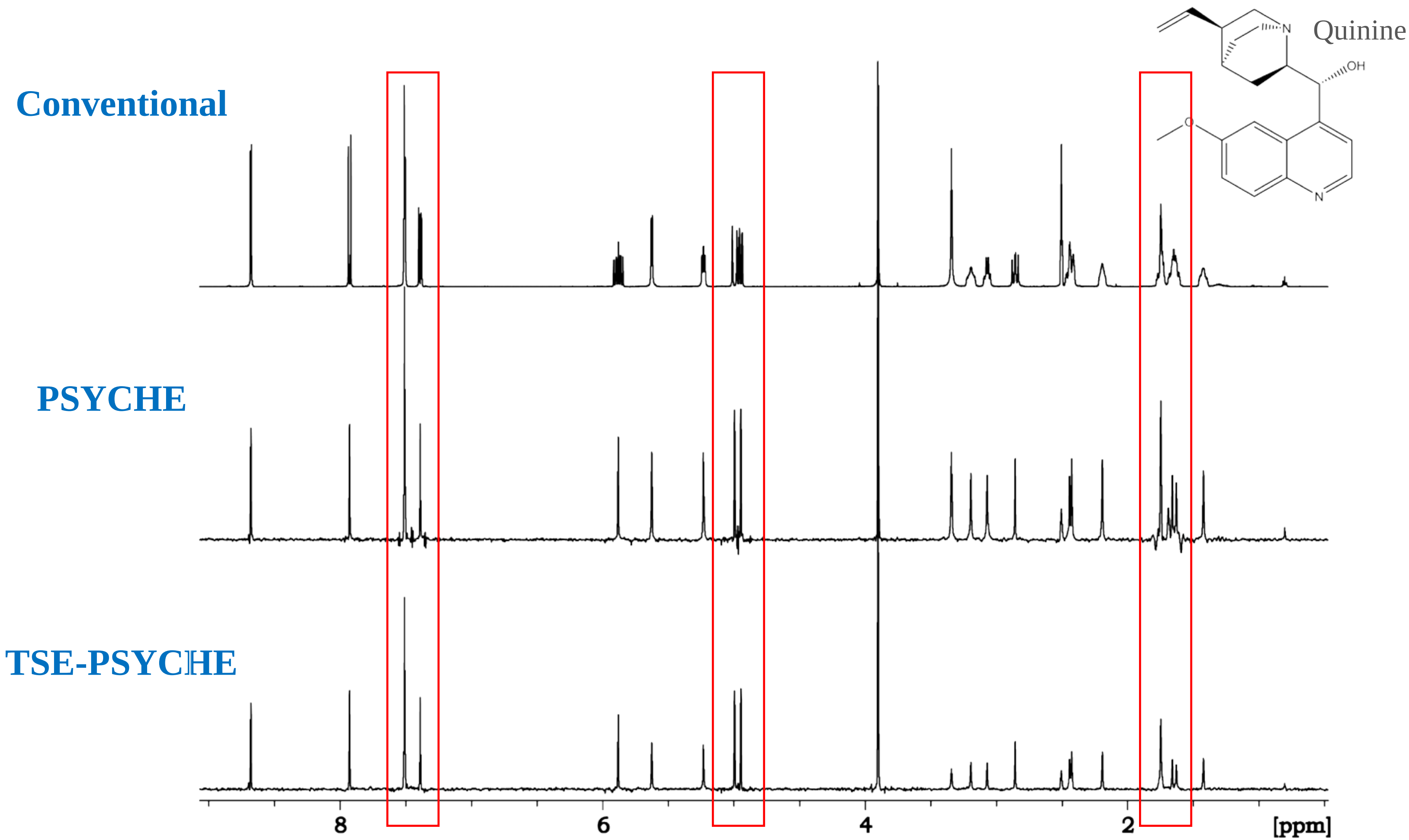
PSYCHE



TSE-PSYCHE

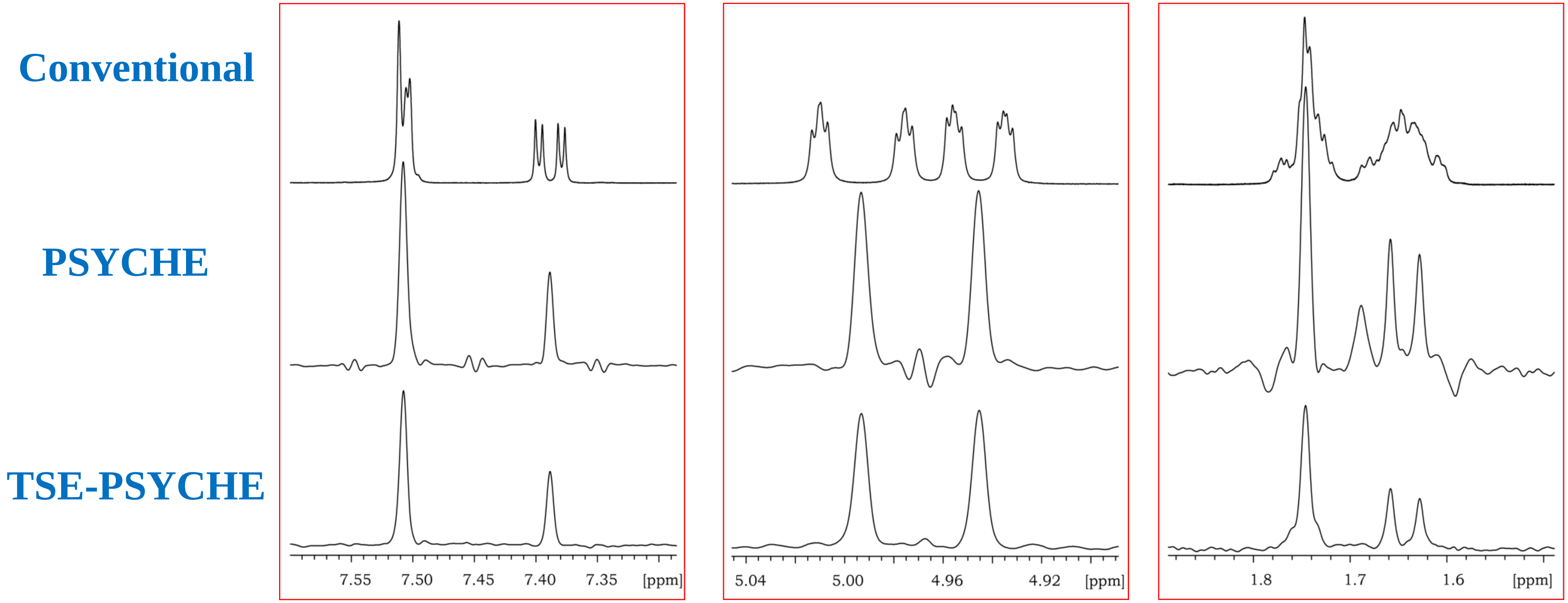


Strong coupling: PSYCHE vs TSE-PSYCHE



Strong coupling: PSYCHE vs TSE-PSYCHE

Generally the signal-to-noise ratio of TSE-PSYCHE can be less than PSYCHE with hard 180° pulse, due to diffusion and relaxation, but the **signal-to-artefact ratio** is much higher



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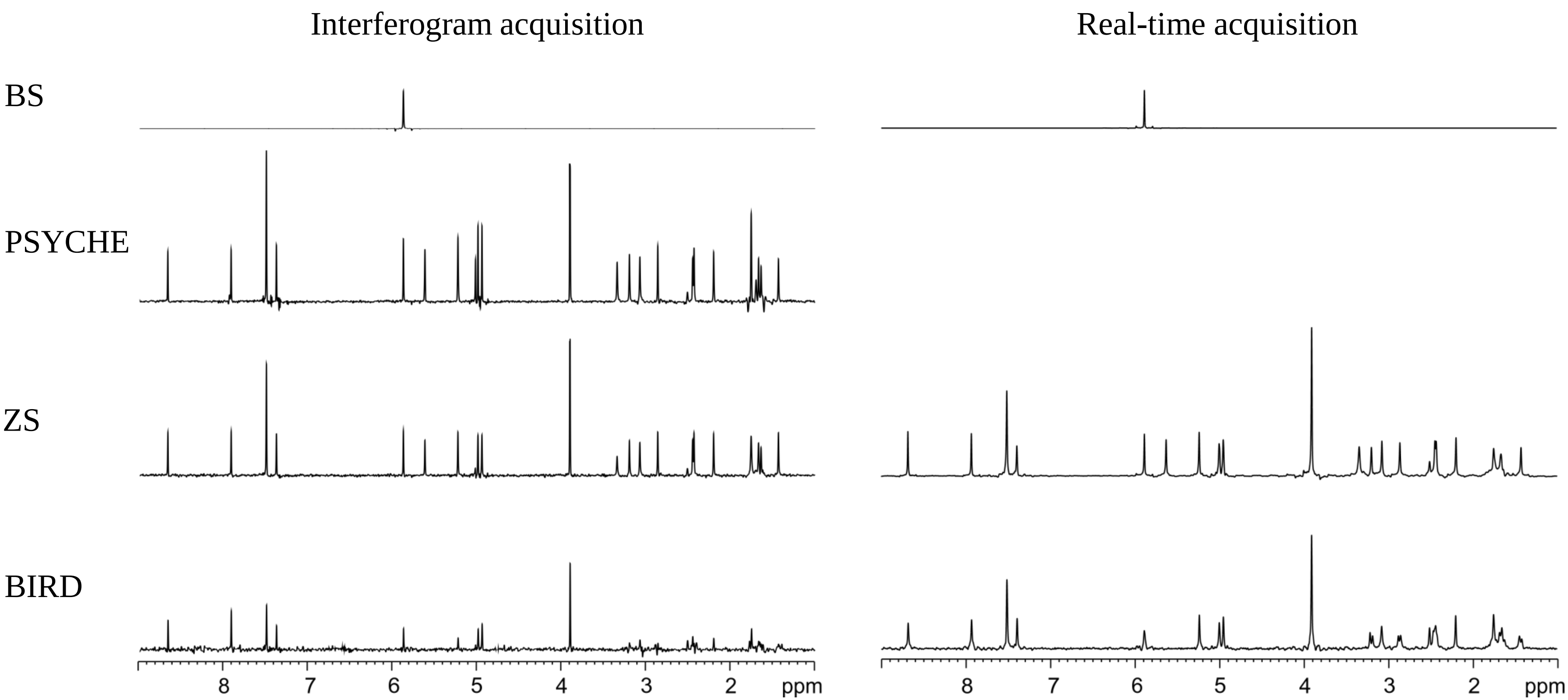

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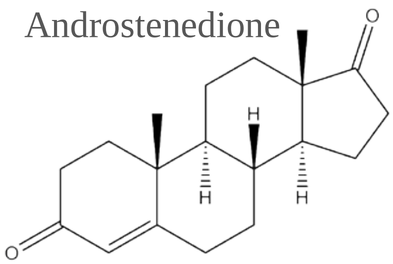
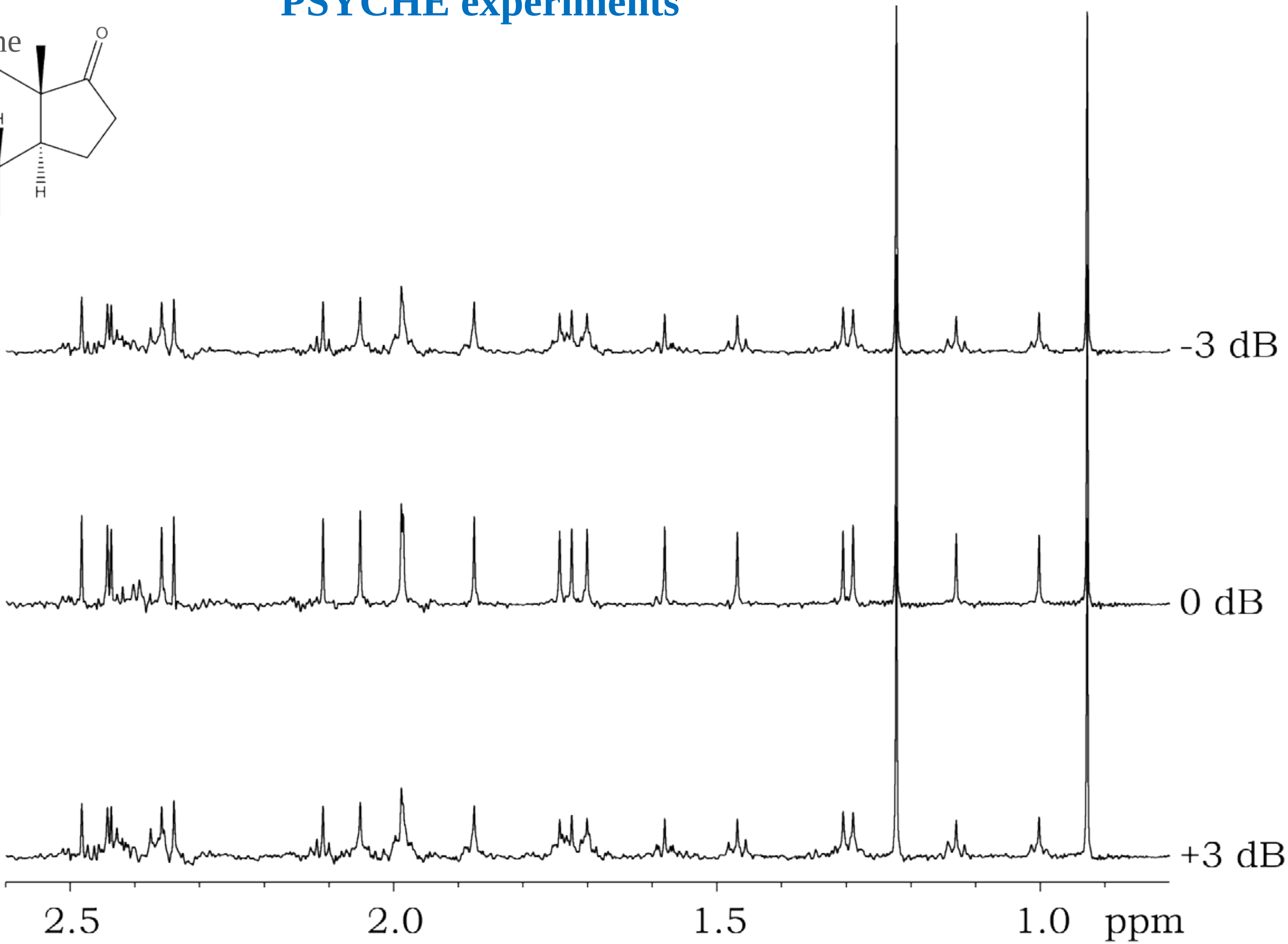
Pulse miscalibration and B_1 inhomogeneity

PSYCHE experiments

Androstenedione



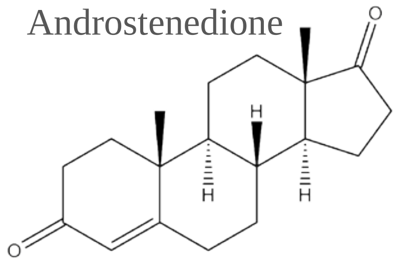


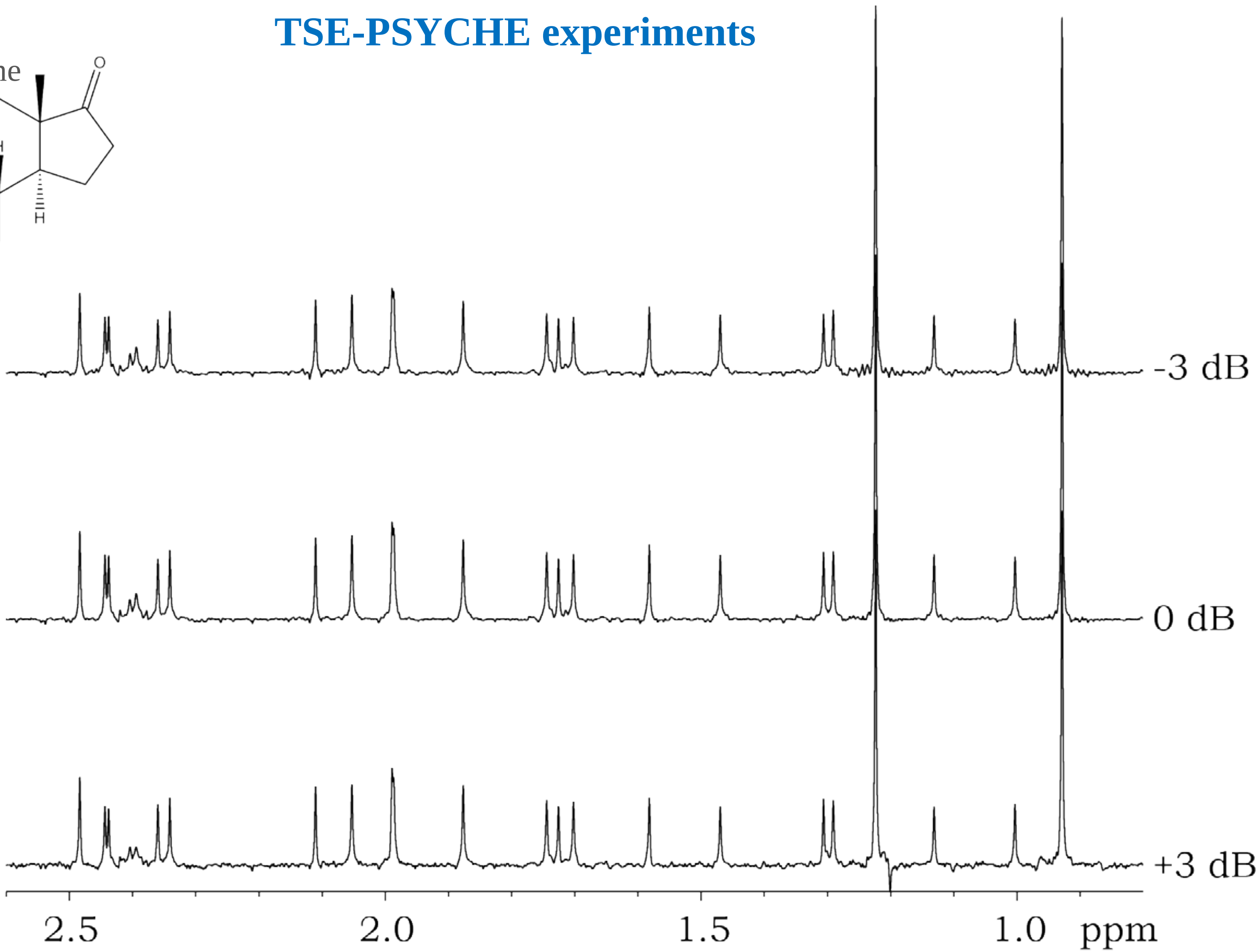
Chem. Commun., **2015**, *51*, 15410.

Pulse miscalibration and B_1 inhomogeneity

TSE-PSYCHE experiments

Androstenedione

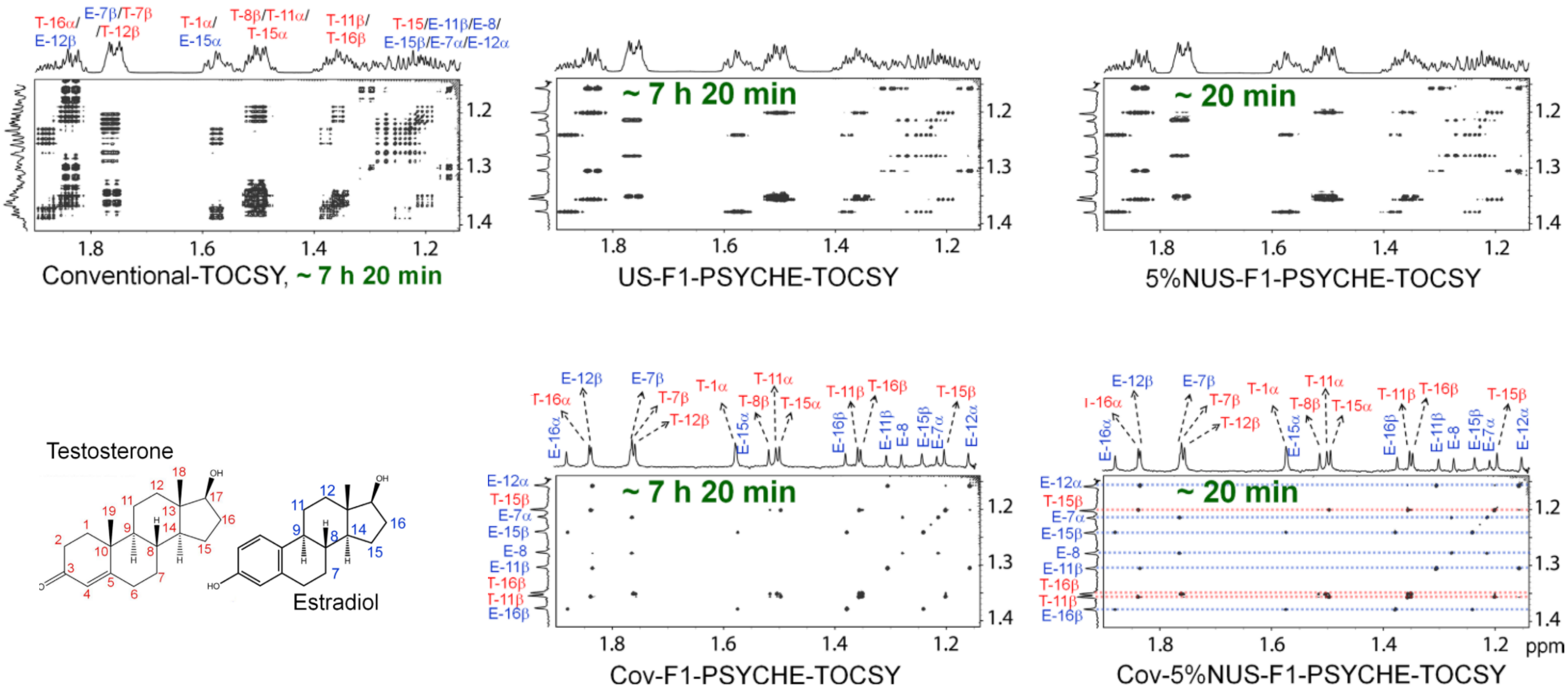




Chem. Commun., **2015**, *51*, 15410.

Speeding things up: non-uniform sampling (NUS)

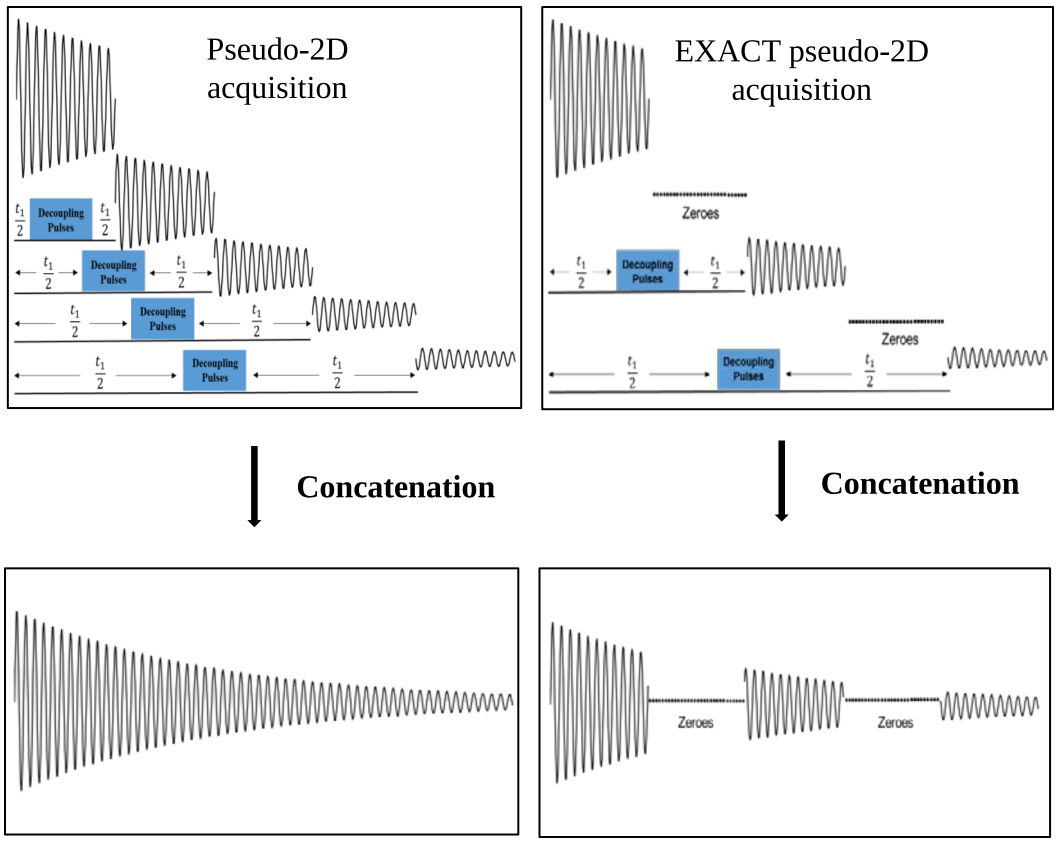
Combining F_1 homodecoupling and NUS



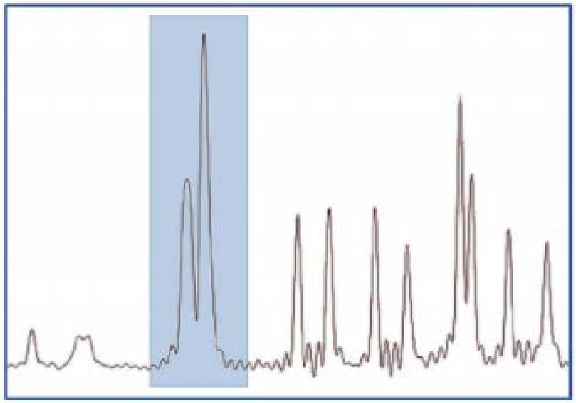
ChemPhysChem. **17**, 2304 (2016)

Speeding things up: non-uniform sampling (NUS)

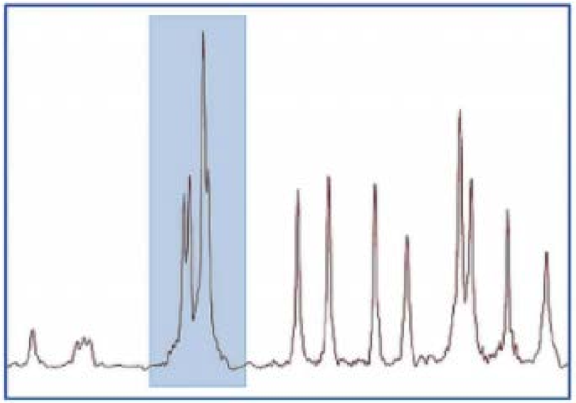
"EXACT" NMR ('burst' non-uniform sampling of data points)



PSYCHE truncated



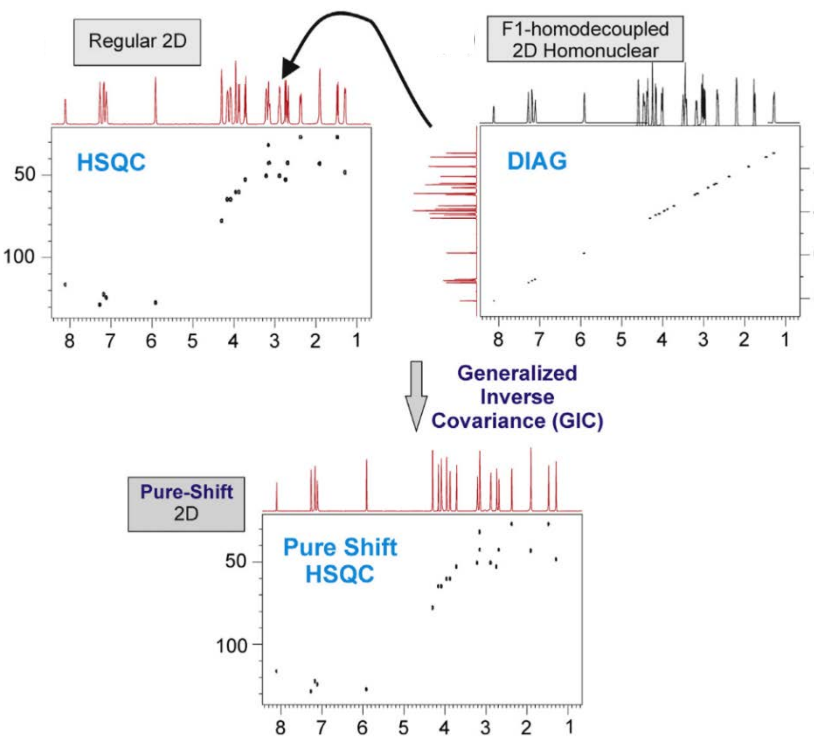
EXACT PSYCHE 37.5%

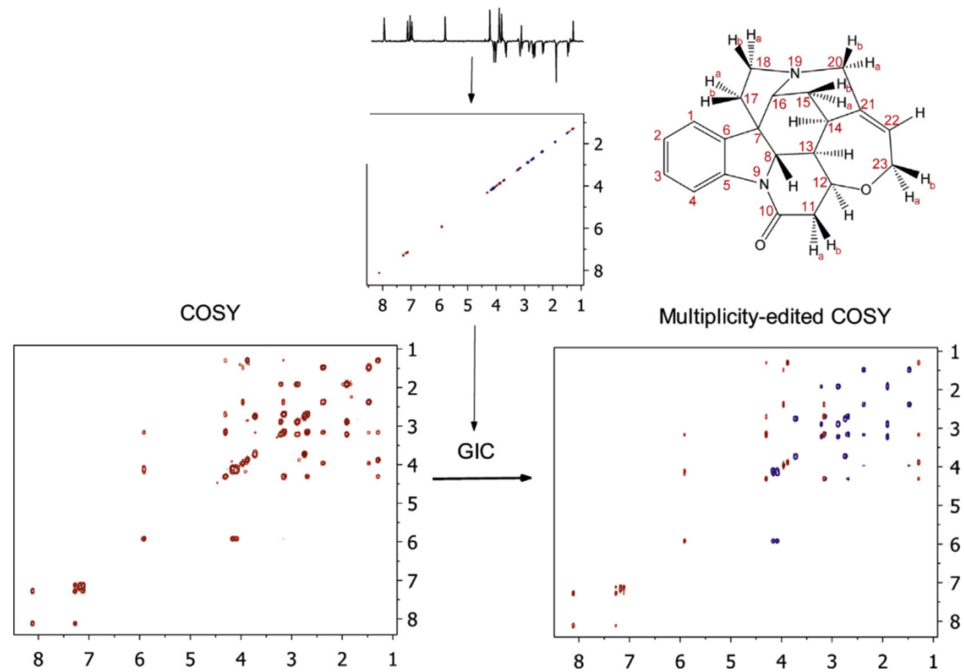


ChemPhysChem. **18**, 2081 (2017)

Combining pure shift experiments with covariance post-processing methods

Using generalize indirect covariance to reconstruct pure shift NMR spectra





Reconstructed homonuclear NMR spectra obtained after indirect covariance processing.

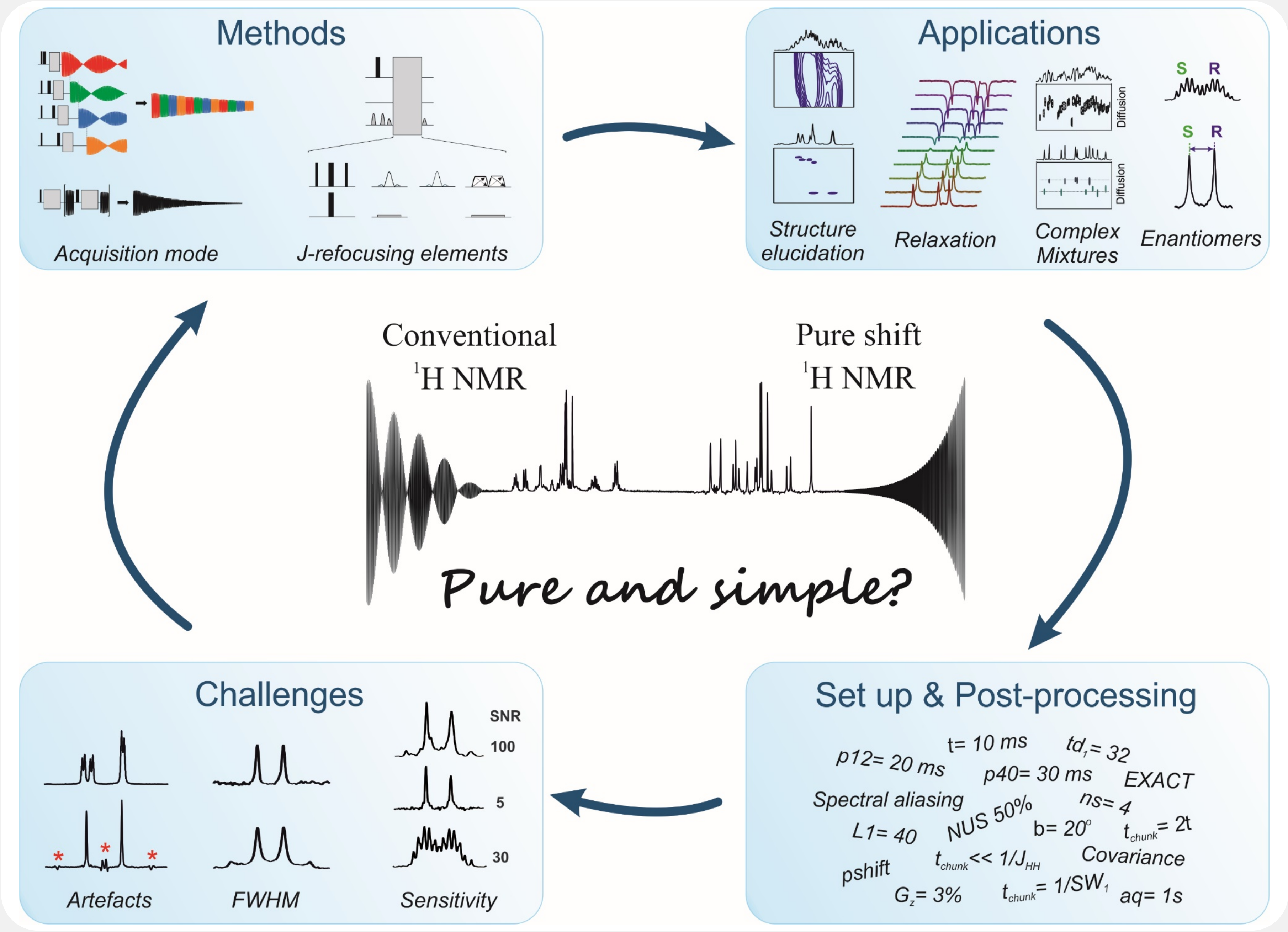
1st component spectrum	2nd component spectrum	Spectrum resulting from the component spectra after covariance processing
COSY	DIAG	**psCOSY**
TOCSY	DIAG	**psTOCSY**
NOESY	DIAG	**psNOESY**
COSY	ME-psDIAG	**ME-psCOSY**
TOCSY	ME-psDIAG	**ME-psTOCSY**

Reconstructed heteronuclear NMR spectra obtained after indirect covariance processing.

1st component spectrum	2nd component spectrum	Spectrum resulting from the component spectra after covariance processing
HSQC-COSY	DIAG	**psHSQC-COSY**
HSQC-TOCSY	DIAG	**psHSQC-TOCSY**
HSQMBC	DIAG	**psHSQMBC**
HSQMBC-COSY	DIAG	**psHSQMBC-COSY**
HSQMBC-TOCSY	DIAG	**psHSQMBC-TOCSY**
ADEQUATE	DIAG	**psADEQUATE**

J. Magn. Reson. **266**, 16 (2016); *J. Magn. Reson.* **270**, 161 (2017)

Summary



Methods
Acquisition mode
J-refocusing elements
Applications
S R
S R
Diffusion
Diffusion
Structure elucidation
Relaxation
Complex Mixtures
Enantiomers
Conventional
1H NMR
Pure shift
1H NMR
Pure and simple?
Challenges
SNR
100
5
30
Artefacts
FWHM
Sensitivity
Set up & Post-processing
t= 10 ms
td1= 32
p12= 20 ms
p40= 30 ms
EXACT
Spectral aliasing
ns= 4
NUS 50%
L1= 40
b= 20°
tchunk= 2t
Covariance
tchunk << 1/JHH
pshift
Gz= 3%
tchunk= 1/SW1
aq= 1s

Acknowledgments

Manchester NMR Methodology Group

Prof. Gareth A. Morris

Dr. Mathias Nilsson

Dr. Mohammadali Foroozandeh

Dr. Peter Kiraly

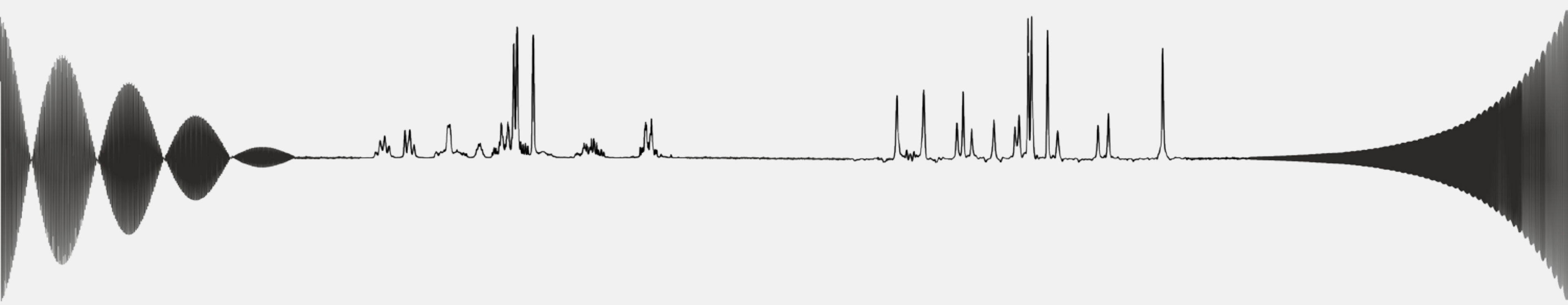
Dr. Ralph Adams

Guilherme Dal Poggetto

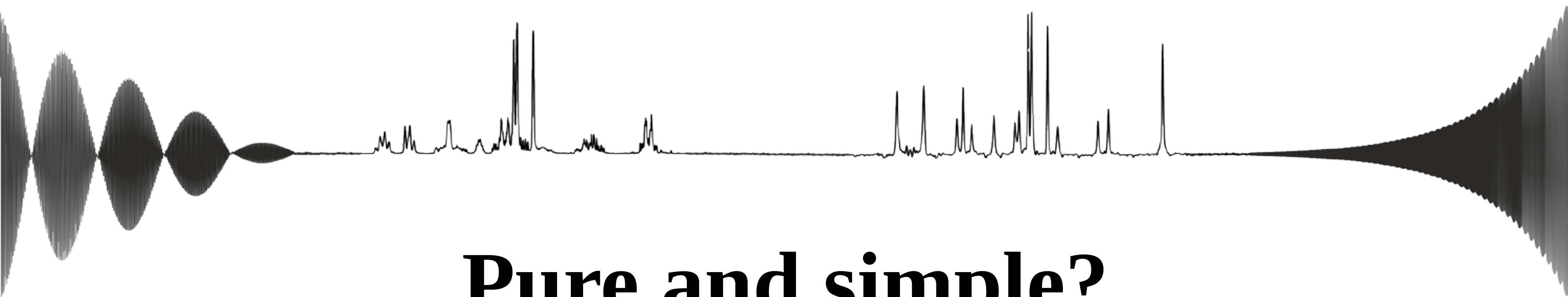
Pinelopi Moutzouri

Aaron Hernandez-Cid

Thank you very much for your attention



Question & answer session



Pure and simple?

Understanding pure shift NMR methodology

Pure shift reviews:

Viva la resolución! Enhancing the resolution of ^{1}H NMR spectra by broadband homonuclear decoupling
N. H. Meyer and K. Zangger, *Synlett*, **2014**, 25, 920-927.

Boosting the resolution of ^{1}H NMR spectra by homonuclear broadband decoupling
N. H. Meyer and K. Zangger, *Chemphyschem*, **2014**, 15, 49-55.

Pure shift NMR spectroscopy
R. W. Adams, *EMagRes*, **2014**, 3, 295-310.

Broadband ^{1}H homodecoupled NMR experiments: recent developments, methods and applications
L. Castañar and T. Parella, *Magn. Reson. Chem.*, **2015**, 53, 399-426.

Pure shift NMR
K. Zangger, *Prog. Nucl. Magn. Reson. Spectrosc.*, **2015**, 86-87, 1-20.

Pure shift ^{1}H NMR: what is next?
L. Castañar, *Magn. Reson. Chem.*, **2017**, 55, 47-53.

Example data: download from our website

http://nmr.chemistry.manchester.ac.uk/pureshift

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Manchester NMR Methodology Group

Home

Workshop on pure shift NMR

Copies of slides for the talks given at the *Workshop on pure shift NMR*, Manchester, 12th Sept 2017 can be accessed via this link.

A data archive containing pure shift pulse sequences, processing software and sample experimental data is available for download via this link.

Workshop on pure shift NMR - slides

Gareth Morris - **Welcome, introduction and history** - pdf - pptx

Peter Kiraly - **Interferogram and real-time acquisition methods** - pdf - pptx

Laura Castañar - **Zangger-Sterk and band-selective methods** - pdf - pptx

Mohammadali Foroozandeh - **PSYCHE** - pdf - pptx - zip including avi videos

Ralph Adams - **Other pure shift and related methods** - pdf - pptx

Mathias Nilsson - **Practical implementation** - pdf - pptx

Adolfo Botana - **JEOL pure shift implementation** - pdf - pptx

Vadim Zorin - **MestreNova pure shift implementation** - pdf - pptx

Ēriks Kupče - **Bruker shaped pulse implementation** - pdf - pptx

Workshop on pure shift NMR - downloads

Data Archives, including instructions, sequences, parameter files and example data

Bruker
Software only (< 1 Mb): Pure_shift_archive_Bruker_software_only.zip.
Full (262 Mb): Pure_shift_archive_Bruker.zip.

Varian
Software only(< 1 Mb): Pure_shift_archive_Varian_software_only.zip.
Full (26 Mb): Pure_shift_archive_Varian.zip.
Manual: UoM_PureShiftNMR_Varian_Manual_rev1.pdf.

External Contributions
DIAG package(< 1 Mb): DIAG_package_Geneva.zip.

The Bruker and Varian/Agilent pure shift data and software archives can also be downloaded from DOI:10.17632/w9nz44cyft.1 and DOI:10.17632/rgj4jwcsnz.1 respectively