Pure Shift NMR

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Tutorial, EUROMAR 2018

Pure Shift NMR

The History: What ? Why ? Who ? When ? How ?

The Mechanics

Some Applications

Some Problems

What *Is* Pure Shift NMR?

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

… in spectra of systems with homonuclear couplings, a perfect pure shift spectrum is an unattainable ideal: all we can do is to approximate it as closely as possible. Strong coupling sets fundamental limits on our ability to distinguish between coupled spins; and practicalities usually force us to compromise between sensitivity and spectral purity. We want methods that are general, robust, linear …

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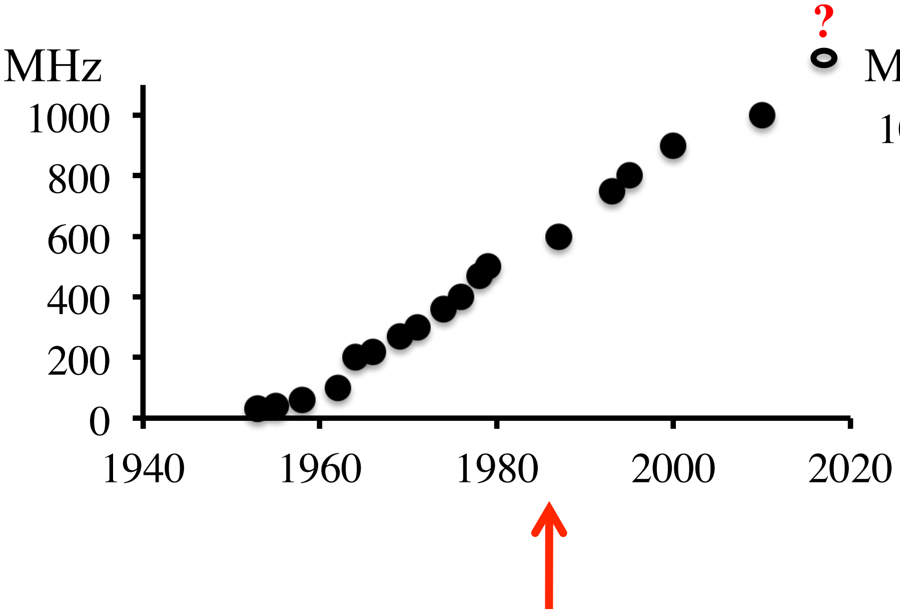
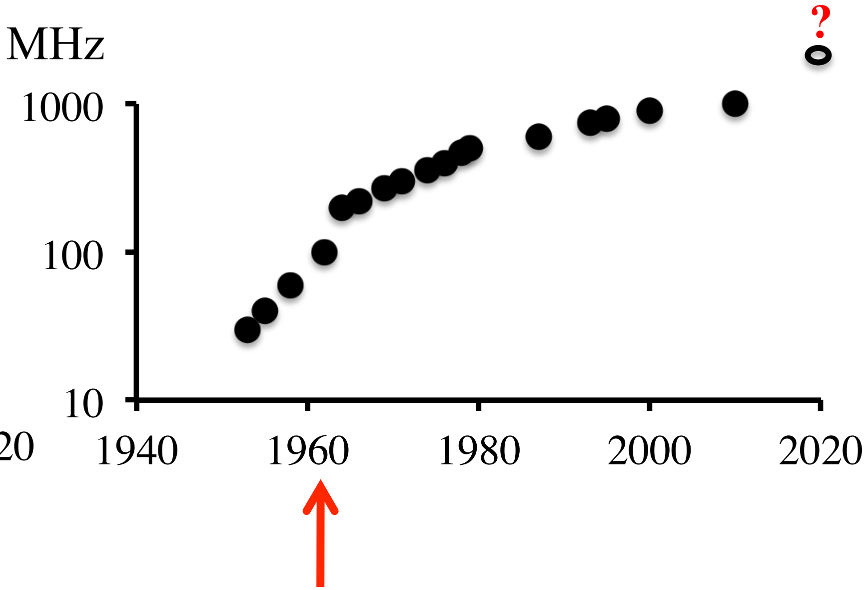
Magnet Development: Proton Resonance Frequency by Year

Maximum commercially available spectrometer field as a function of year, on linear (left) and log (right) scale





Introduction of superconducting magnets

Is High Temperature Superconductivity the Answer?

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1962: Richard Ernst's PhD project …

"… My own work dealt with the construction of high sensitivity radio frequency preamplifiers … on the theoretical side, I was concerned with stochastic resonance … **I tried in particular to design a scheme of homonuclear broadband decoupling to simplify proton resonance spectra. By applying a stochastic sequence with a shaped power spectral density that has a hole at the observation frequency, all extraneous protons should be decoupled without perturbing the observed proton spin.** The theoretical difficulties were mainly concerned with the computation of the response to nonwhite noise. Experiments were not attempted at that time, we did not believe in the usefulness of the concept anyway, and I finished my thesis in 1962 with a feeling like an artist balancing on a high rope without any interested spectators."

Richard Ernst, Nobel autobiography (1991)

1976: 45° Projection of a 2D *J* Spectrum

Homonuclear broad band decoupling and two-dimensional *J*-resolved NMR spectroscopy*

W. P. Aue, J. Karhan, and R. R. Ernst

Laboratorium für Physikalische Chemie, Eidgenössische Technische Hochschule, 8006 Zürich, Switzerland
(Received 2 March 1976)

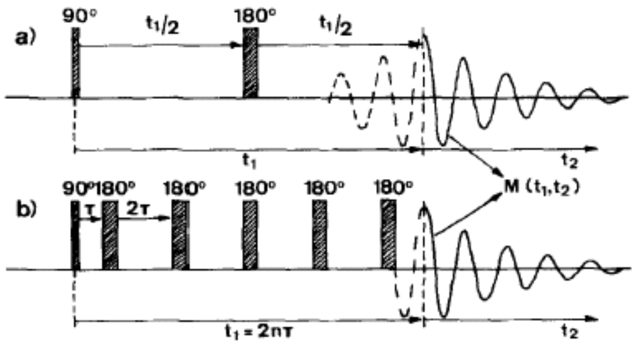
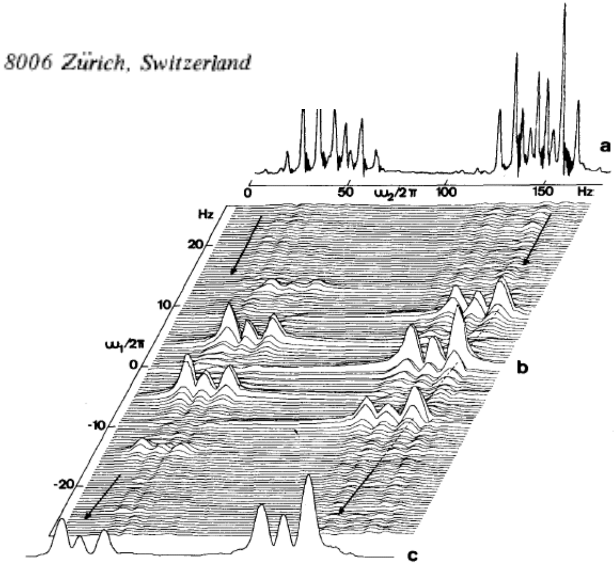


FIG. 1. Two basic schemes for 2D *J*-resolved spectroscopy. (a) Single echo experiment. (b) Spin echo sequence. Except for the effects of diffusion, both experiments produce the same results.



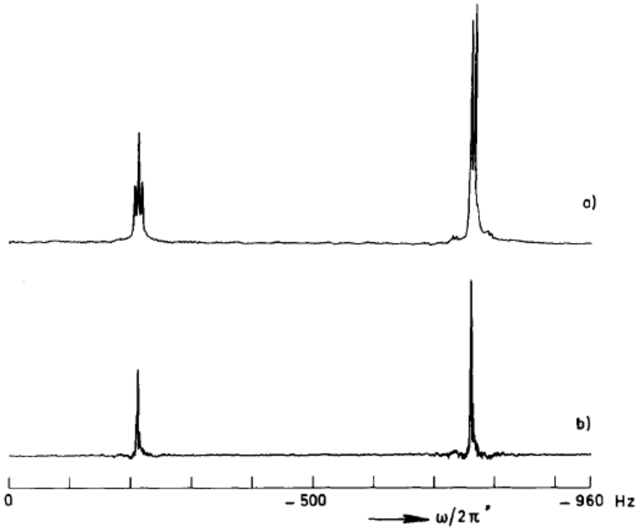
Projecting the 2D*J* spectrum of a weakly coupled spin system at 45° to the F_2 axis generates a pure shift spectrum, but only if it is the absolute value spectrum that is projected (which loses most of the resolution gain)

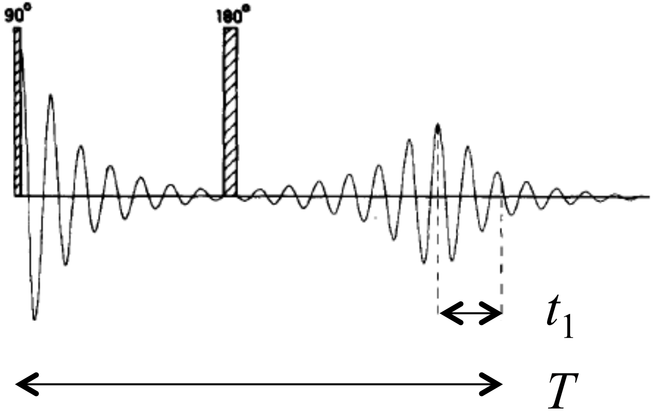
J Chem Phys **64**, 4229 (1976)

1979: Constant-Time Evolution

Homonuclear Broadband-Decoupled Absorption Spectra, with Linewidths Which Are Independent of the Transverse Relaxation Rate

A. BAX
A. F. MEHLKOPF
J. SMIDT





J Magn Reson **35**, 167 (1979)

Varying t_1 maps out an FID that only depends on chemical shift, but signal amplitudes oscillate as a function of T

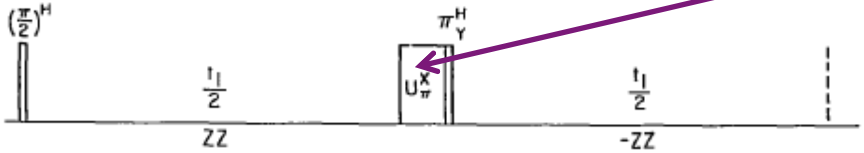
1982: BIRD

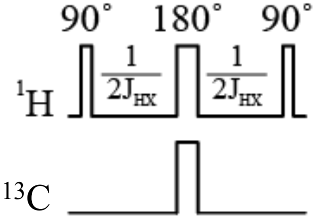
BILINEAR ROTATION DECOUPLING OF HOMONUCLEAR SCALAR INTERACTIONS

J.R. GARBOW, D.P. WEITEKAMP and A. PINES

Department of Chemistry and Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720, USA

Received 1 September 1982; in final form 5 October 1982





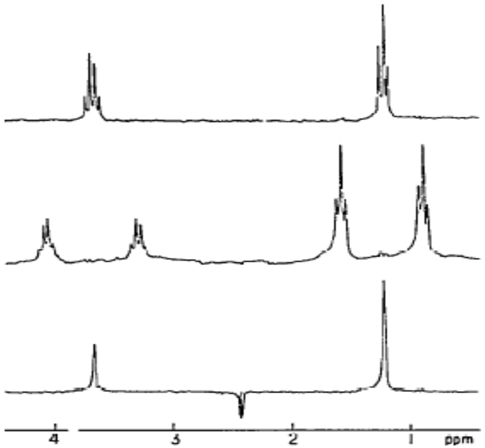


Fig. 1. Proton NMR spectra at 182 MHz of ethanol illustrating removal of *J*-couplings by BIRD. Samples were spun horizontally at 60–80 Hz in a vertical superconducting magnet with a horizontal solenoid r.f. coil.

Using a BIRD sequence element that inverts only ^{13}C-attached protons and a hard 180° pulse at the midpoint of t_1 refocuses the effects of J_{HH}. *(First example of a "J-refocused" pure shift experiment)*

Chem Phys Lett **93**, 504 (1982)

1997: Pattern Recognition in Symmetrised 2D *J* Spectra

Proton Chemical-Shift Spectra

SVETLANA SIMOVA,* HELMUT SENGSTSCHMID,† AND RAY FREEMAN

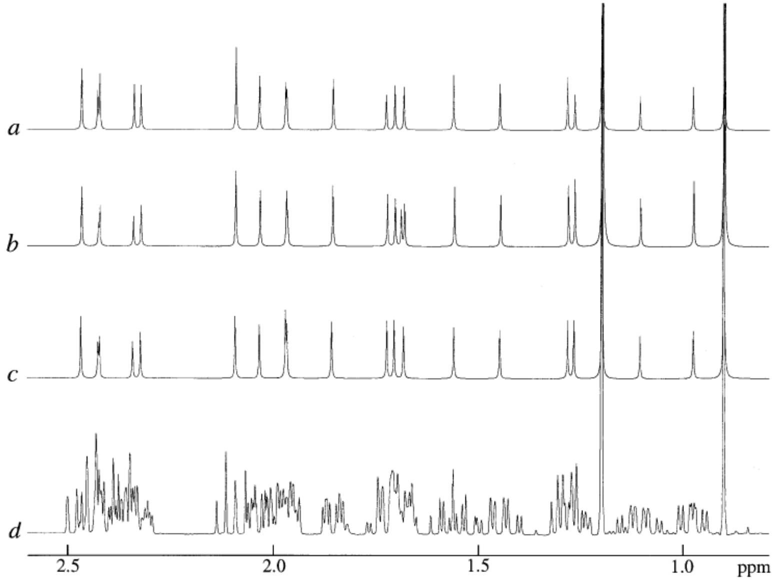


FIG. 15. Chemical-shift spectra of 4-androsten-3,17-dione obtained (a) from the reflected *J* spectrum, (b) from the purged *J* spectrum, and (c) from the *z*-filtered *J* spectrum. There is an additional response in (b) near 1.7 ppm from the residual water signal. The conventional spectrum (d) is included for comparison.

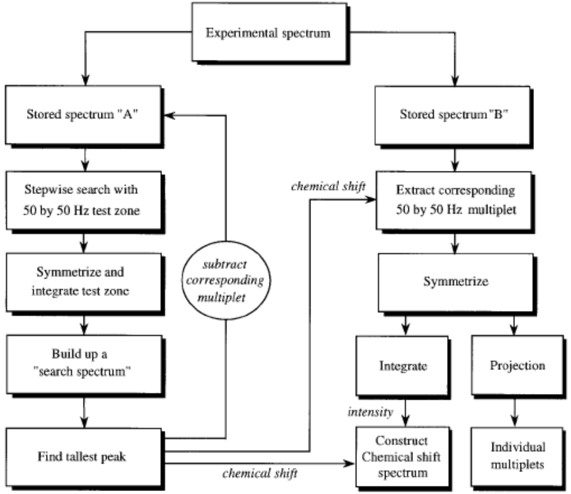


FIG. 5. Schematic flow chart of the program to extract chemical shifts and individual spin-multiplet patterns. Note that a second copy "B" of the experimental spectrum is used for the final processing stage; this avoids cumulative errors arising from repeated subtraction.

J Magn Reson **124**, 104 (1997)

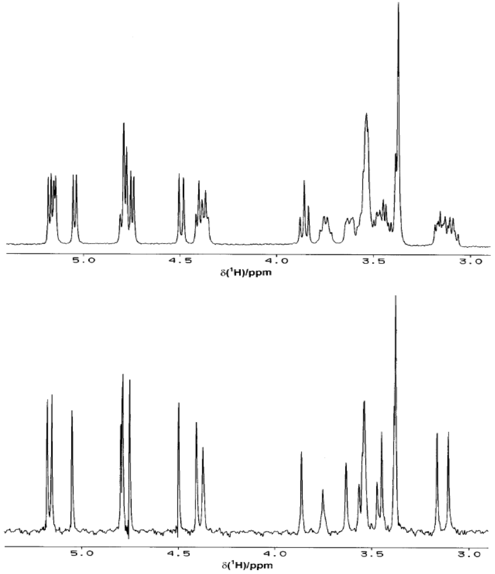
2D*J* spectroscopy with a *z*-filter gives cross-shaped multiplets; nonlinear pattern recognition processing converts these into a pure shift spectrum

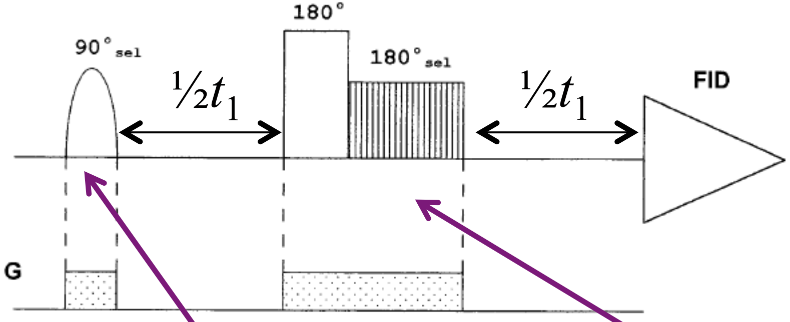
1997 : the Zangger-Sterk Method

Homonuclear Broadband-Decoupled NMR Spectra

KLAUS ZANGGER AND HEINZ STERK*

Institut für Organische Chemie, Karl-Franzens-Universität Graz, Heinrichstraße 28, 8010 Graz, Austria





Replacing the BIRD sequence element with a selective 180° pulse in the presence of a field gradient refocuses *J* in thin slices of the sample.

Observing only these slices gives a pure shift spectrum.

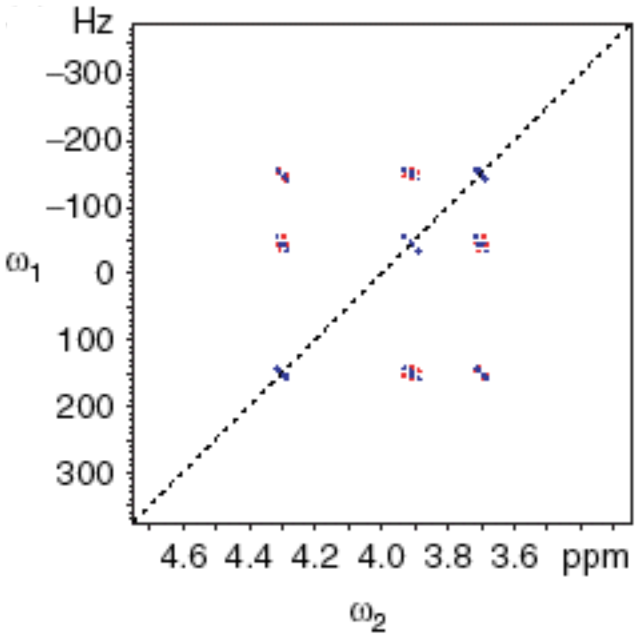
(First example of "chunked" pure shift data acquisition)

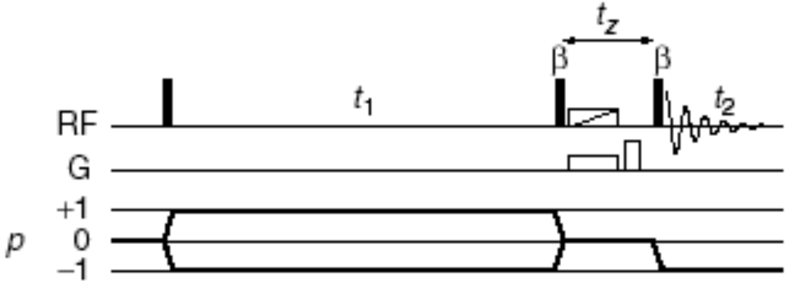
J Magn Reson **124**, 486 (1997)

2007 : Anti-z-COSY

Broadband proton-decoupled proton spectra†

Andrew J. Pell,[1] Richard A. E. Edden[2] and James Keeler[1]*





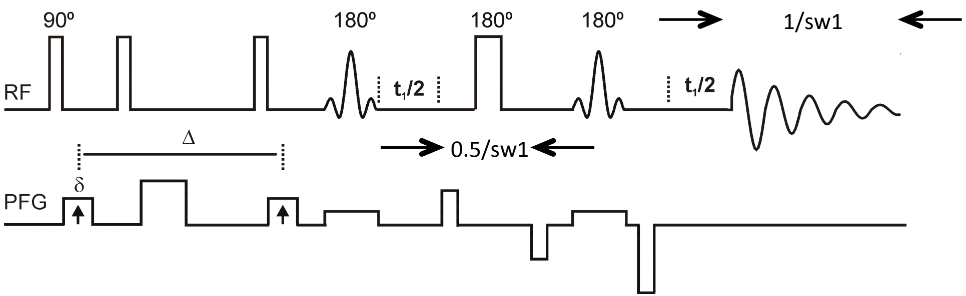
A *z*-filtered anti-z-COSY mixing period restricts multiplet components to those at right angles to the diagonal; filtering out cross-peaks and projecting in this direction gives a pure shift spectrum

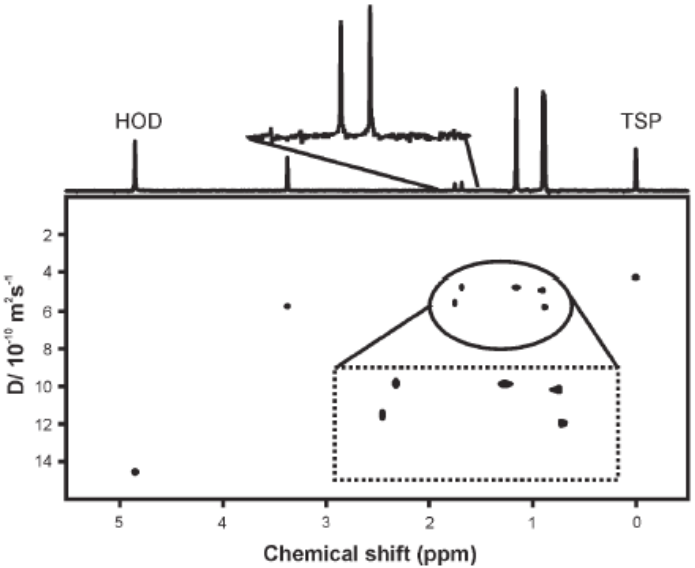
Magn Reson Chem **45**, 296 (2007)

2007 : Zangger-Sterk Revisited / Pure Shift DOSY

Pure shift proton DOSY: diffusion-ordered ^{1}H spectra without multiplet structure

Mathias Nilsson and Gareth A. Morris*





Using pure shift acquisition avoids signal overlap which would otherwise lead to incorrect apparent *D* values.
(Updated the ZS method to use PFGs, and changed chunk timing)

Chem. Commun. **2007**, 933 (2007)

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Pure Shift NMR: Broadband Homonuclear Decoupling

$$\hat{\mathcal{H}} = -\sum_i \nu_i \hat{I}_z^i + \sum_{i<j} J_{ij} \underline{\hat{I}}^i \cdot \underline{\hat{I}}^j \simeq -\sum_i \nu_i \hat{I}_z^i + \sum_{i<j} J_{ij} \hat{I}_z^i \hat{I}_z^j$$

Shifts

Couplings

We need a way to separate the effects of shifts (δ) and couplings (J), e.g.

- hard 180° pulse
  reverses effects of δ but not of J

- J-refocusing
  reverses effect of J but not of δ, e.g. by combining "active spin refocusing" (reverse δ for chosen spins only) with a hard 180° pulse (inverts all spins)

Pure Shift NMR

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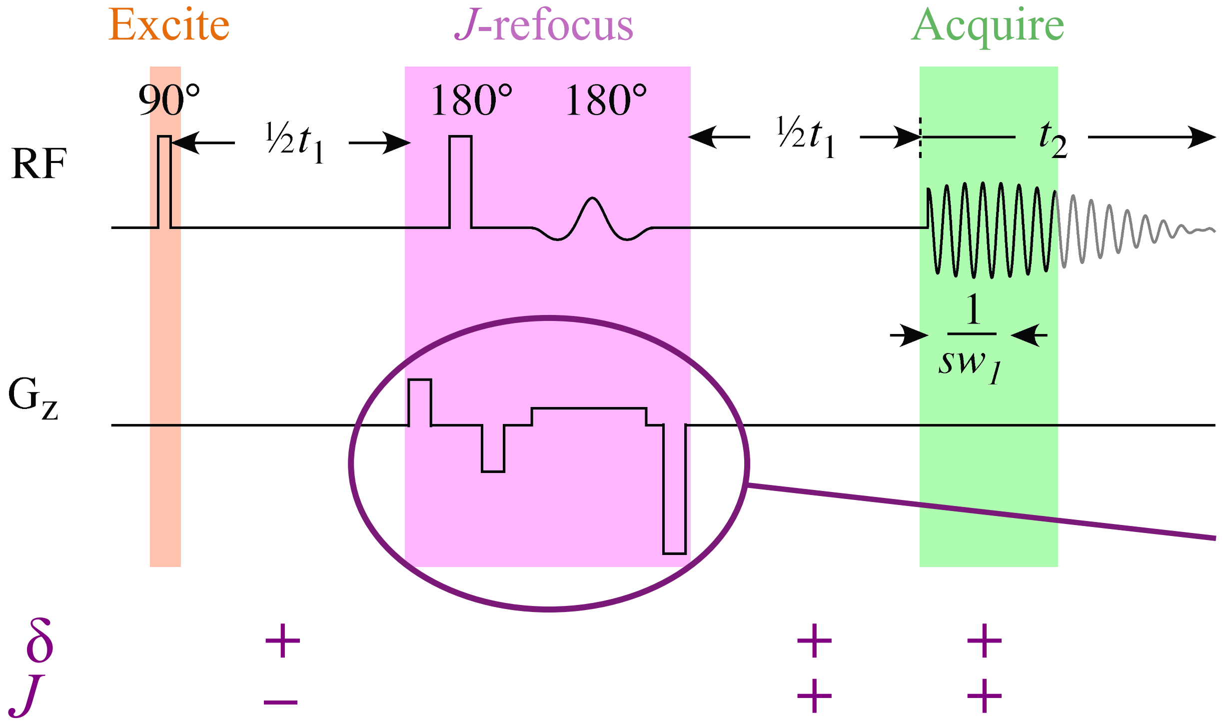
The Mechanics:

the Zangger-Sterk experiment, *J*-refocusing and PSYCHE

Some Applications

Some Problems

J-Refocusing: the Zangger-Sterk Sequence Revisited



Using gradient pulses to enforce CTPs cleans up results and can remove the need for a slice-selective 90°pulse

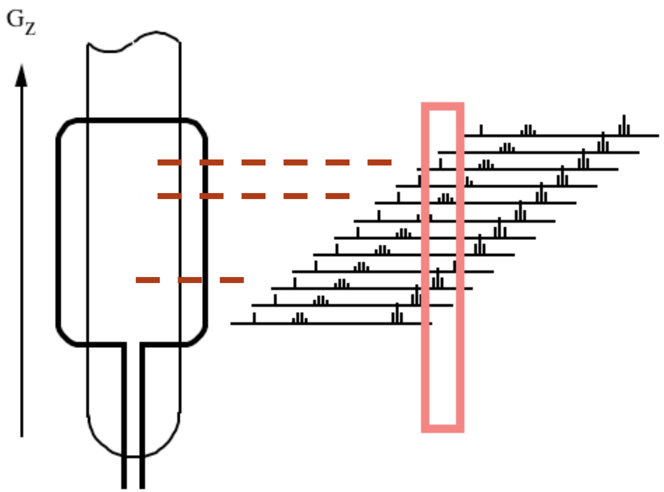
The combination of a hard 180° pulse and a slice- and shift-selective 180° pulse leaves the active spins (within the slice) unperturbed and the passive (outside the slice or at a different shift) inverted, refocusing the *J* modulation of the active spins.

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

Chem. Commun. **2007**, 933

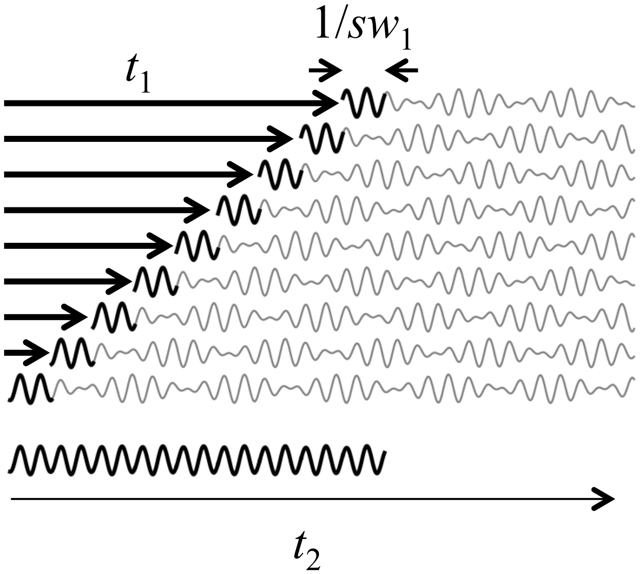
Mechanics of the Zangger-Sterk Experiment

J-refocusing



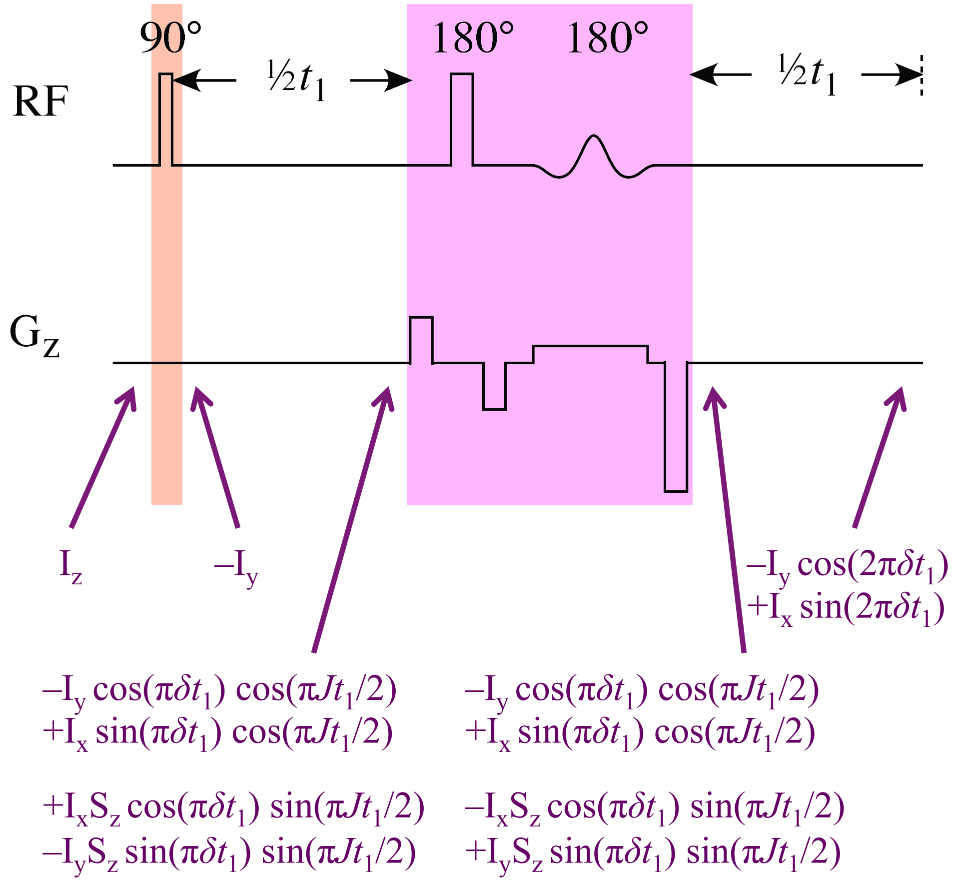
The soft and hard 180° pulses invert the passive spins, refocusing *J* modulation but leaving shift evolution intact

Data chunking



J modulation is slow, so a block of data points lasting $1/sw_1 << 1/J$ can be measured for each value of t_1, building up a pure shift interferogram. (The residual effect of *J* is to cause weak sidebands at multiples of sw_1).

Taking the Zangger-Sterk Sequence Apart (1)



Maps out signal dependence on t_1, as in a 2D experiment

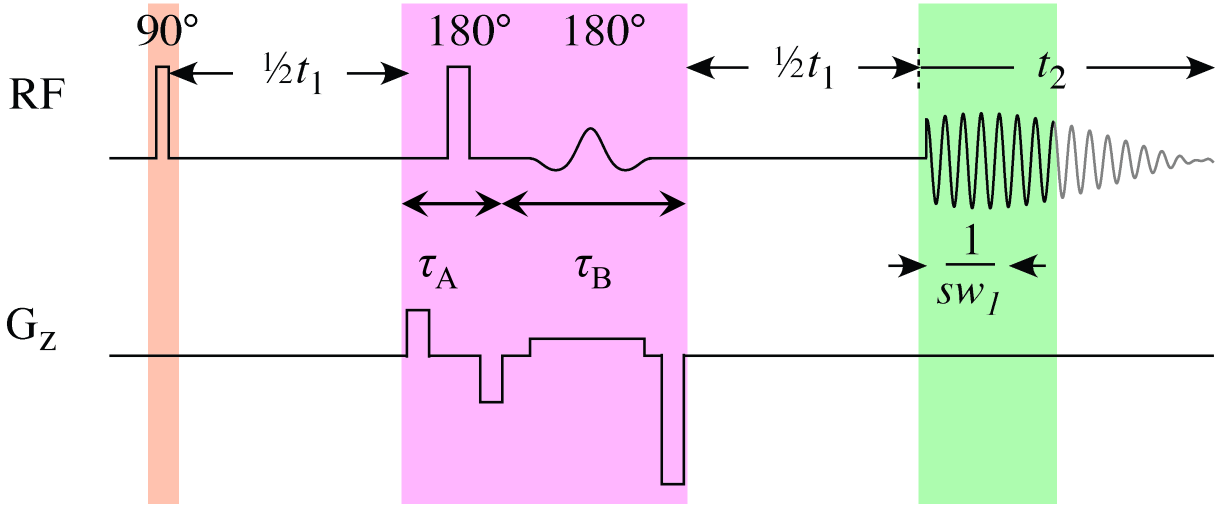
The soft 180° pulse with the weak field gradient inverts each resonance in a different thin slice of the sample

The strong gradient pulses select spins inverted by both 180° pulses, so the only signals seen are from the thin slices of sample – the "**active spins**"

The active spins experience both 180° pulses, so do not have their shift refocused

The remaining, "passive", spins are left inverted, so their coupling effect on the **active** spins is reversed – "*J*-refocusing"

Taking the Zangger-Sterk Sequence Apart (2)



The hard 180° pulse refocuses δ, but J continues to evolve for a time τ_A

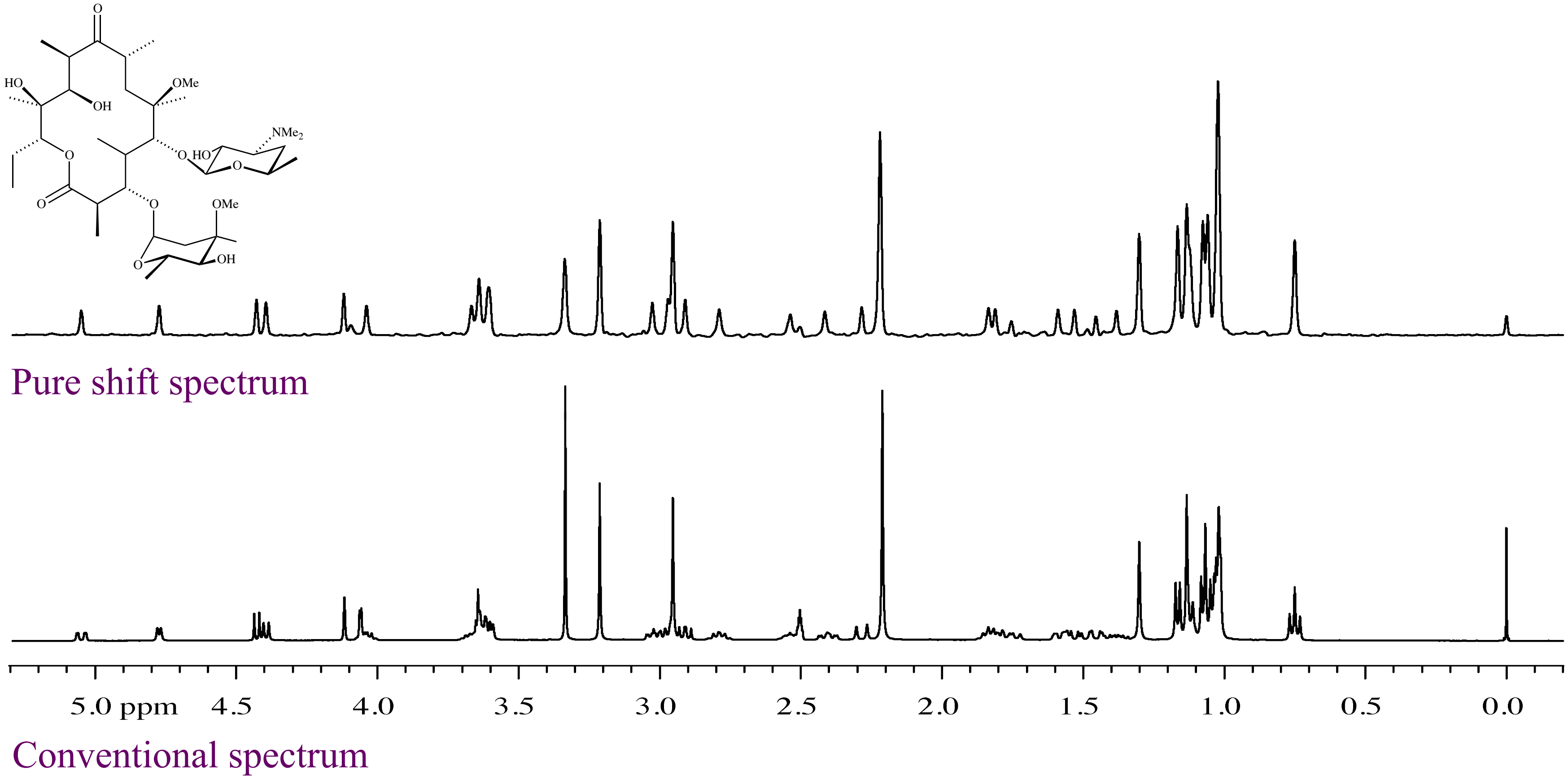
The soft 180° pulse affects only one spin at a time, simply refocusing signals, so τ_B has no effect on shift or J evolution

The net effect is that J refocuses at a time $t_1 + 1/(2\ sw_1)$, at the midpoint of the chunk of data

In the original ZS experiment J was refocused at the end of t_1. Refocusing $\tau_A = 1/(2\ sw_1)$ later means that twice as long a chunk of data can be acquired without increasing the effect of J modulation

(In practice, a few extra data points are acquired before the start of the chunk, but then thrown away, in order to avoid the distortions caused by receiver switch on and digital filtration)

400 MHz Zangger-Sterk Pure Shift ^1H Spectrum of Clarithromycin

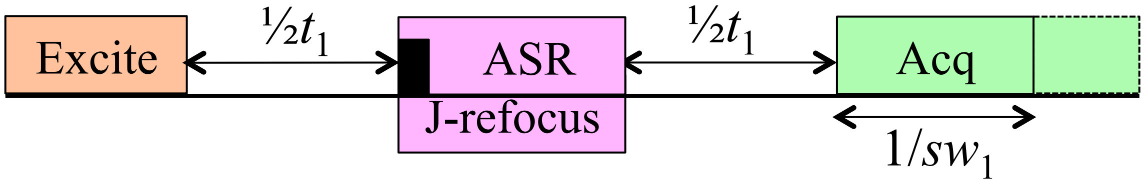


J Am Chem Soc **132**, 12770 (2010)

J-Refocused Pure Shift NMR: Acquisition Methods

Interferogram

(2D acquisition to build up an interferogram)



J is refocused by the combination of a hard 180° pulse and an active spin refocusing (**ASR**) sequence element (in the ZS experiment, a soft pulse under a field gradient) at the midpoint of t_1. A pure shift interferogram is built up by incrementing t_1 in steps of 1/*sw1*. *Slow but sure.*

Real-time

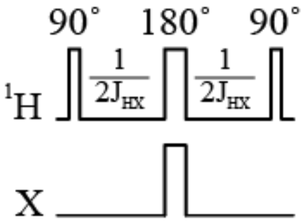
(acquire an FID in real time)

In real-time experiments, acquisition of a FID is periodically interrupted by J-refocusing elements. *Fast, but relaxation and other effects broaden the pure shift signals.*

(An intermediate alternative is semi-real-time acquisition – *J Magn Reson* **293**, 19-27 (2018))

Pulse Sequence Elements for "Active Spin Refocusing"

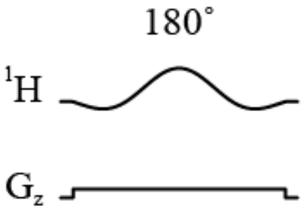
We divide the available spins into *active* spins that we observe, and *passive* spins that we manipulate. Combining an ASR element with a hard 180° pulse refocuses the effect on the active spins of any couplings to the passive spins.



BIRD

BIlinear Rotation Decoupling:

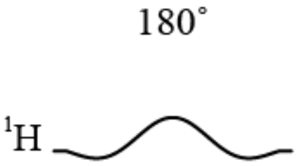
π rotation of protons coupled to ^{13}C



ZS

Zangger-Sterk:

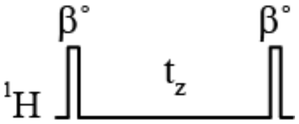
slice- / shift-selective π rotation



BS

Band-Selective homonuclear decoupling:

shift-selective π rotation

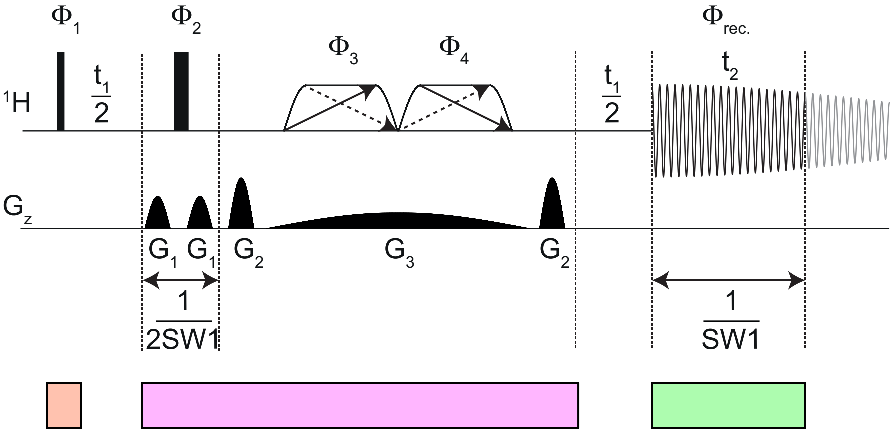


"double β" (PSYCHE)

PSYCHE / anti-z-COSY:

π rotation of a fraction $\sin^2\beta$ of spins

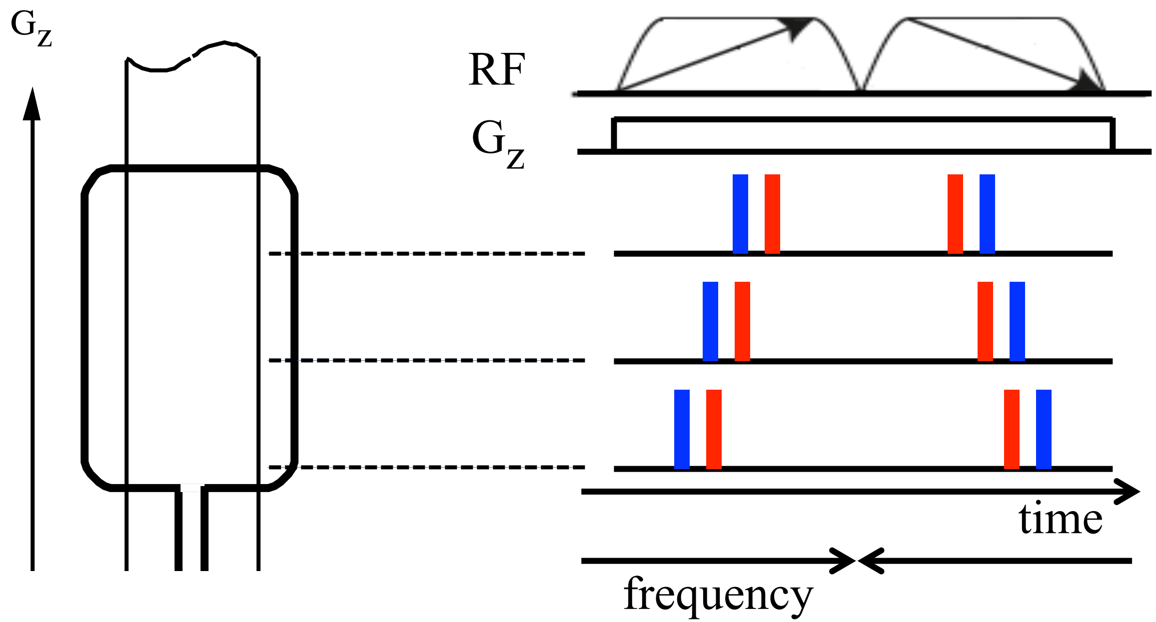
PSYCHE: Pure Shift Yielded by Chirp Excitation



Using two small flip angle β swept-frequency (chirp) pulses, with opposite sweep directions and under a field gradient, preserves only the subset of coherences ("diagonal peak" responses) that form a stimulated echo without changing frequency.

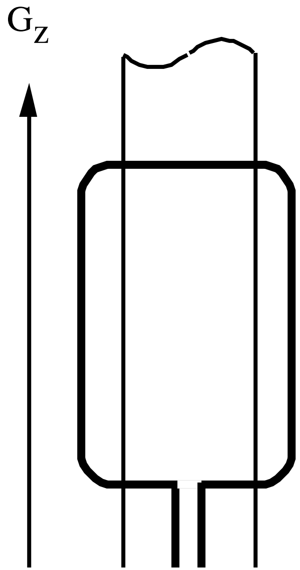
Angew Chem Int Ed **53**, 6990 (2014)

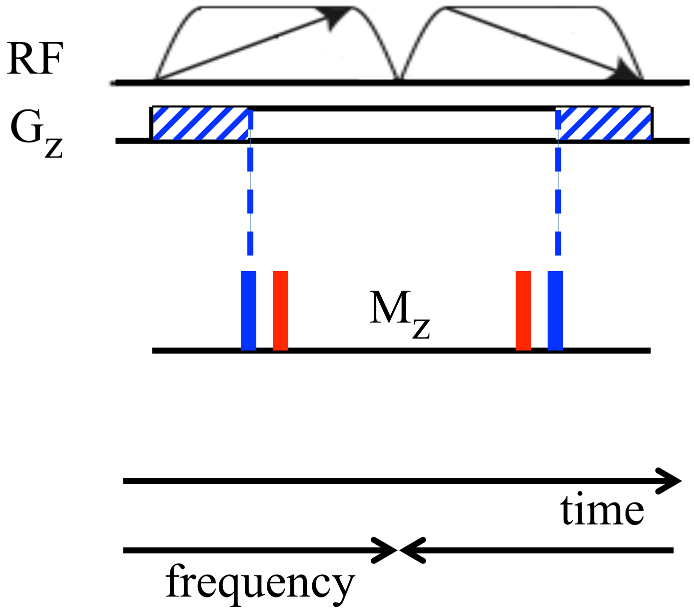
Mechanism of PSYCHE (1)



Consider two coupled spins **A** and **X**. In the presence of a field gradient, the times at which **A** and **X** are at resonance during the chirp pulses will vary with position, so we can treat each slice as experiencing a series of four small flip angle pulses β.

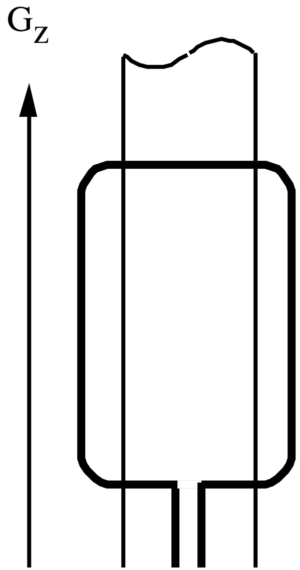
Mechanism of PSYCHE (2)

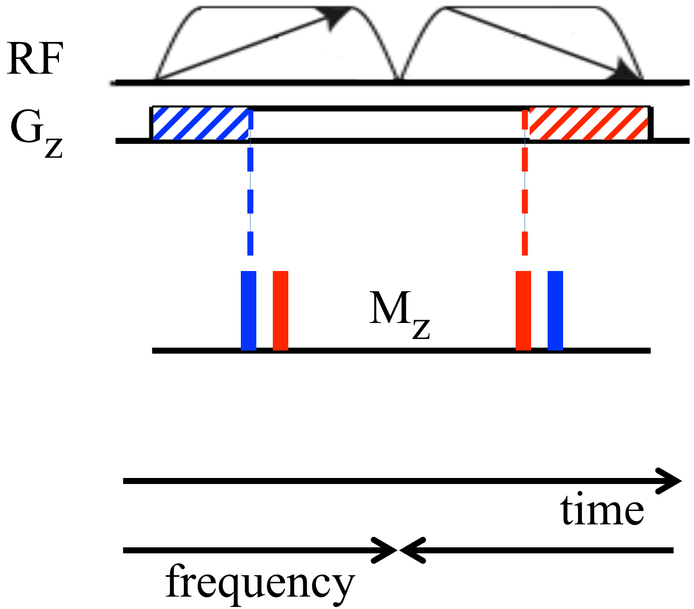




"Diagonal peak" responses are refocused, and survive

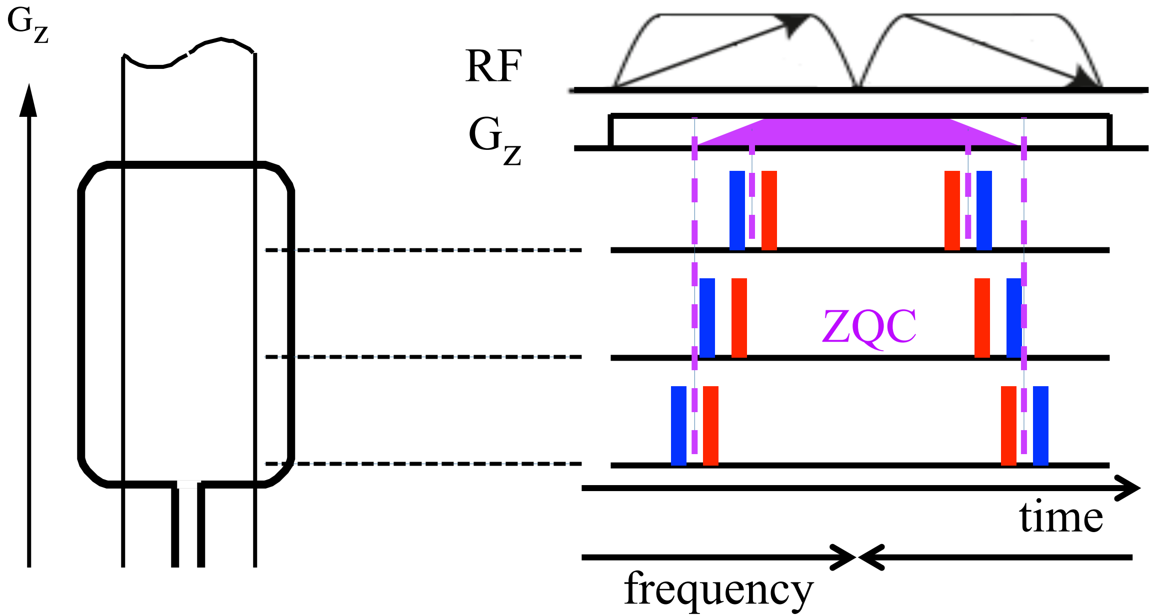
Mechanism of PSYCHE (3)





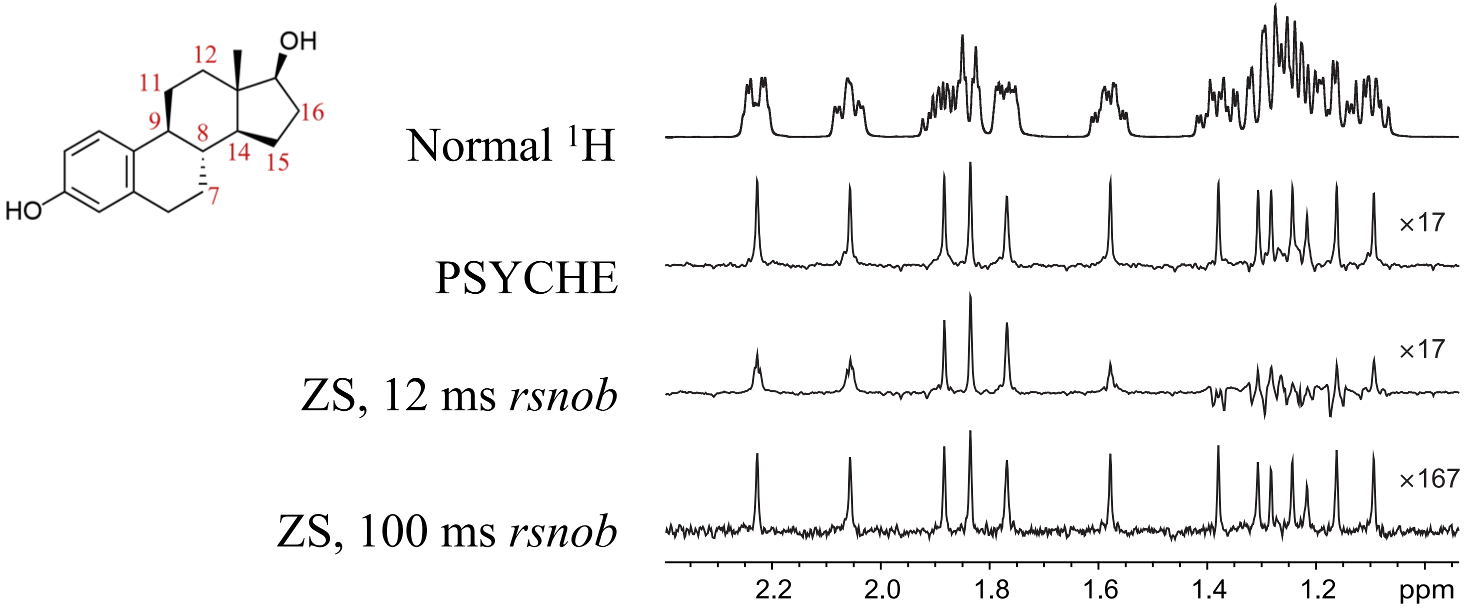
"Cross-peak" responses are dephased

Mechanism of PSYCHE (4)



Zero quantum coherence pathways experience different evolution times in different slices, and average to zero

PSYCHE Sensitivity and Spectral Purity



Comparing PSYCHE and ZS methods for a complex and strongly coupled region of the 500 MHz ^{1}H spectrum of estradiol, PSYCHE offers ca. 10× more S/N for similar spectral purity. In ZS, spectral purity is determined by selective pulse bandwidth; in PSYCHE, by β. (The sensitivity of ZS methods can be improved by using multiple frequency irradiation – nemo-ZS.)

The decoupled signal in PSYCHE $\propto 2\sin^2\beta\cos^2\beta$, the main ("recoupling") artefact $\propto \sin^4\beta$.

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

Pure Shift NMR

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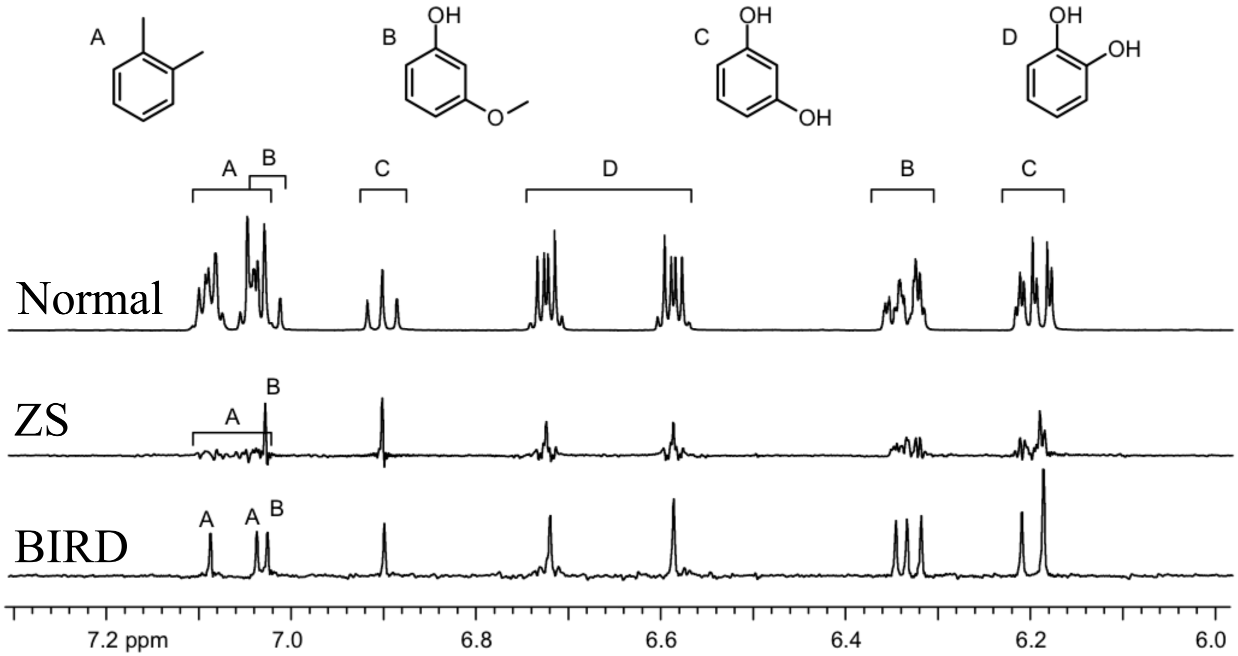
Mechanics

Applications

Problems

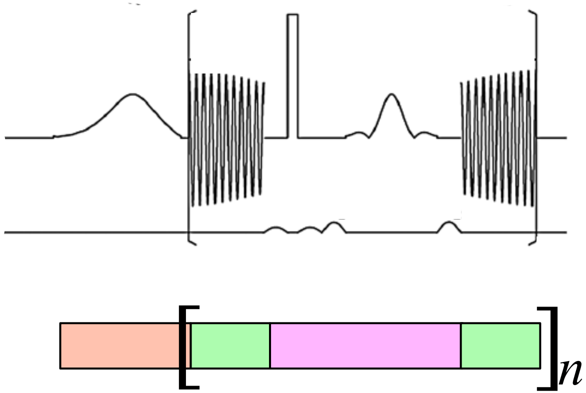
BIRD Pure Shift NMR of Strongly Coupled Aromatics

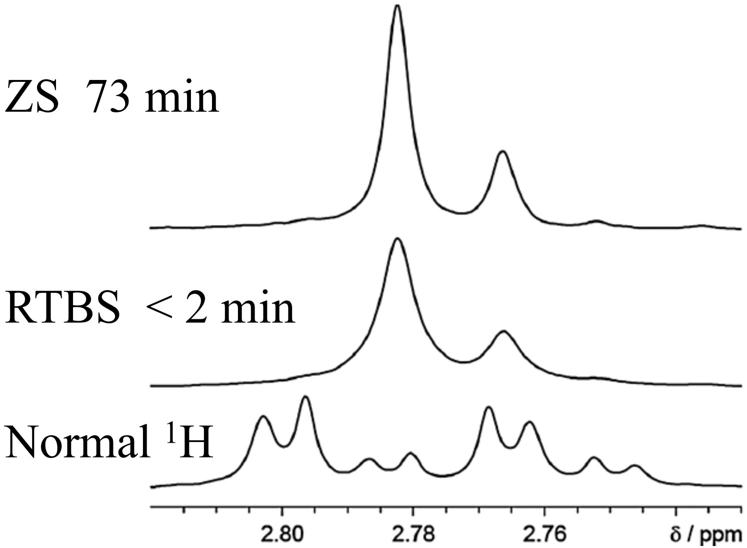


Selecting only those protons directly bonded to ^{13}C can lift ^{1}H degeneracy and restore weak coupling.

Angew Chem Int Ed **50**, 9716 (2011)

Real-time BS Pure Shift NMR of Hesperidin Diastereomers



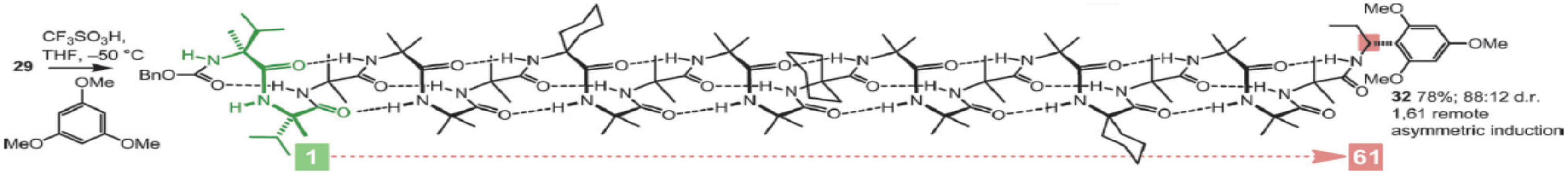


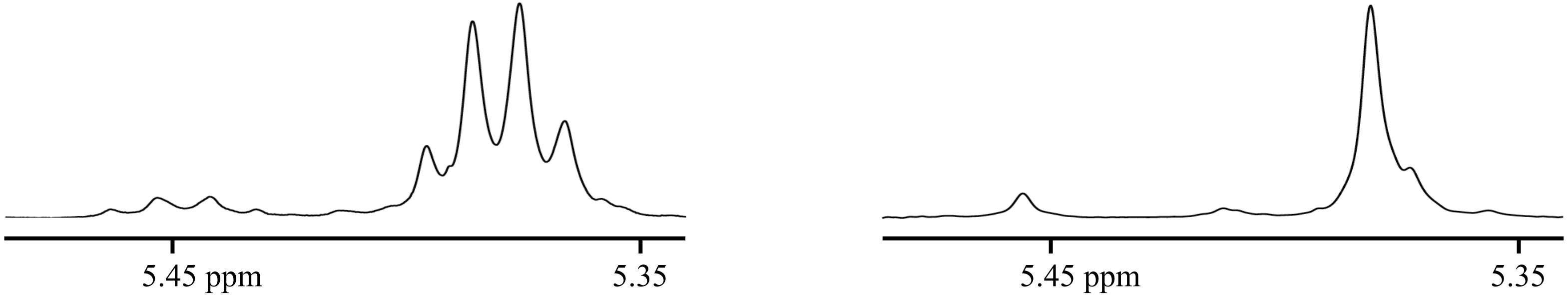
Periodically inverting all except those protons within a specified frequency band – band-selective homonuclear decoupling, BASHD – suppresses the effects of couplings to protons within that band.

Chem Commun **50**, 2512 (2014)

VIP Remote Stereocontrol Very Important Paper
DOI: 10.1002/anie.201308264

Foldamer-Mediated Remote Stereocontrol: > 1,60 Asymmetric Induction**

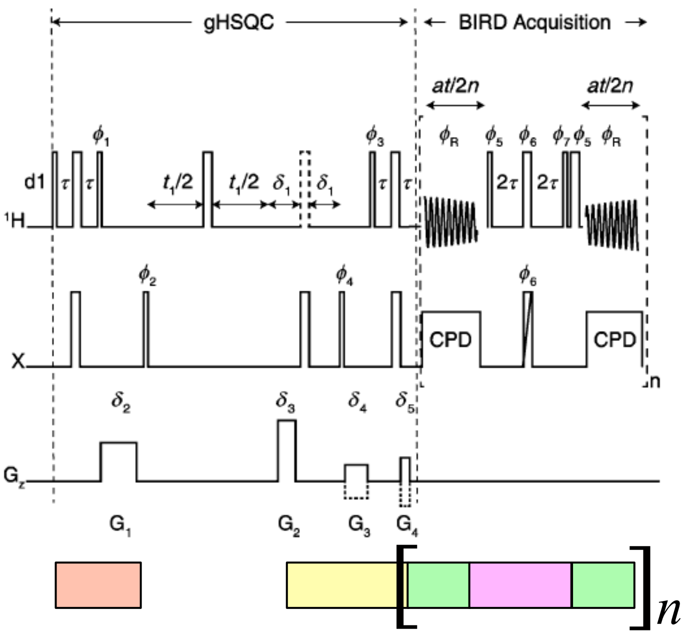


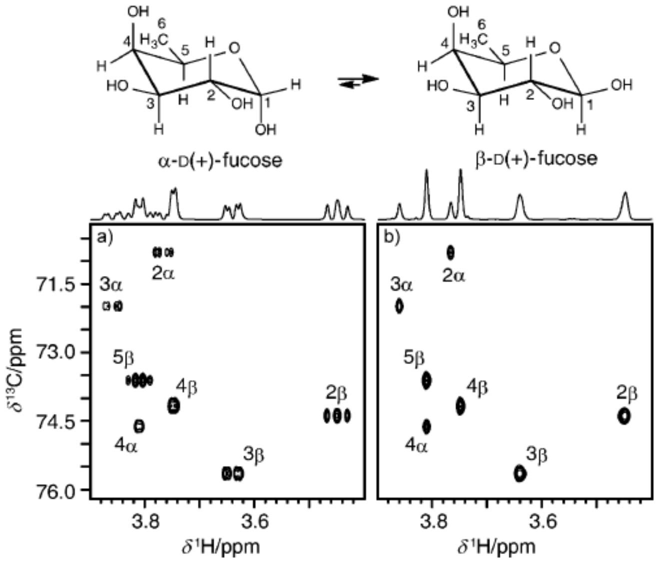


An 800 MHz real-time band-selective pure shift spectrum allows the enantiomeric excess induced at the end of the peptidomimetic chain to be determined reliably.

Angew Chem Int Ed **53**, 151 (2014)

Real-Time BIRD Pure Shift HSQC

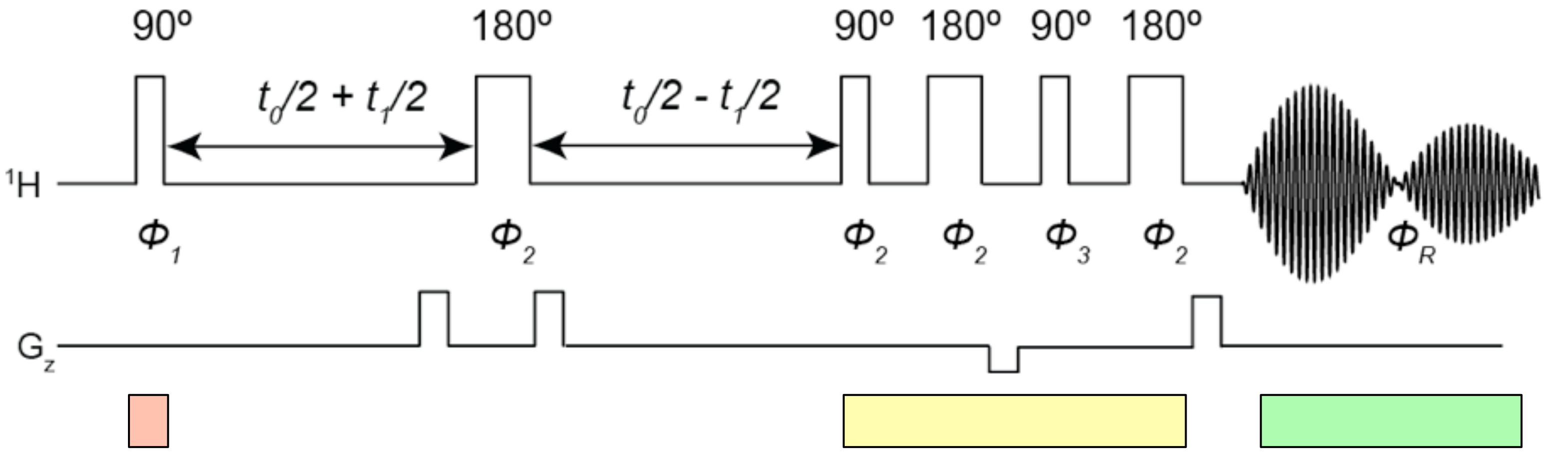




Periodic application of BIRD/180° sequence elements during data acquisition suppresses the effects of ^{1}H-^{1}H homonuclear couplings in real time. Unusually, pure shift here increases both resolution and sensitivity.

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

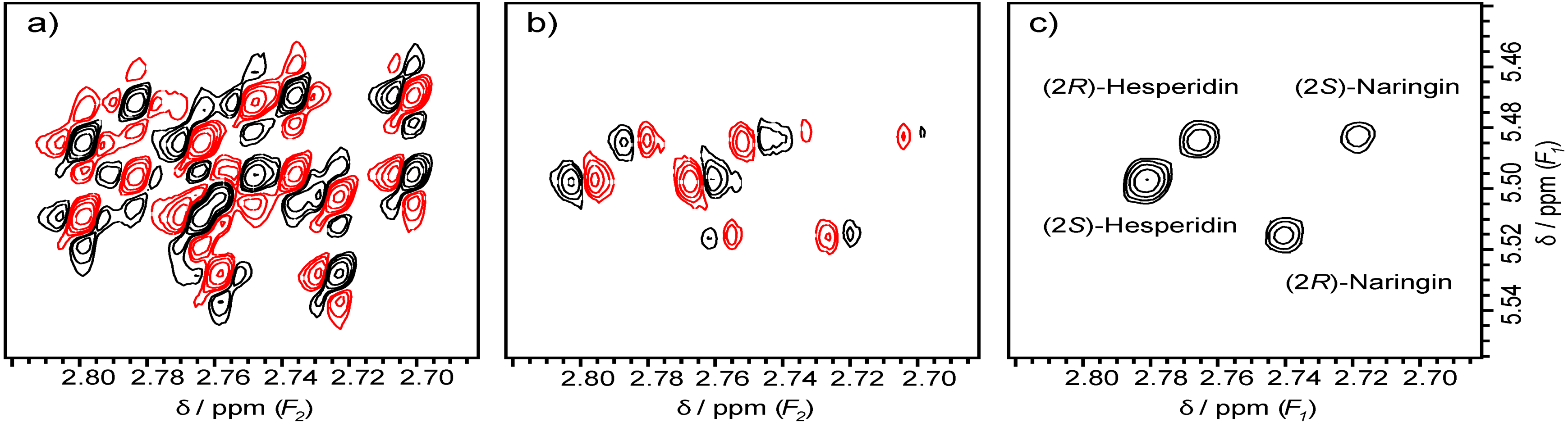
Pure shift CT-nQF-COSY



2D data are acquired conventionally, with constant time t_0 chosen to maximise cross-peaks. 2DFT gives an F_1-pure shift nQF-COSY, which can be covariance processed to yield a double pure shift nQF-COSY spectrum.

Angew Chem Int Ed **51**, 6460 (2012)

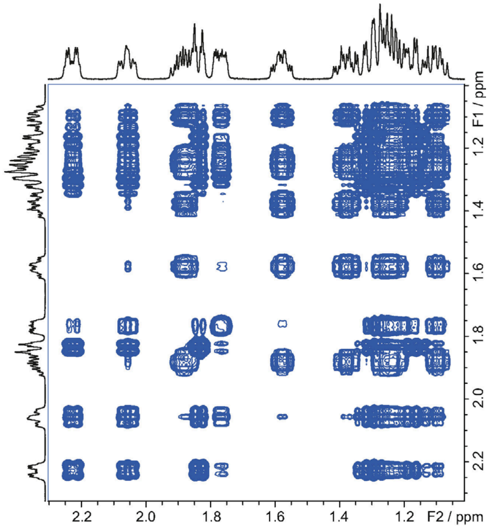
500 MHz pure shift CT-3QF-COSY: flavonoids



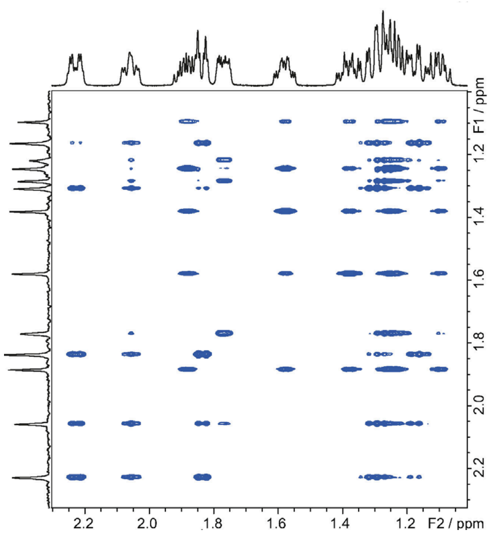
Normal 3QF-COSY (a), CT-3QF pure shift COSY (b), and covariance double pure shift CT-3QF-COSY (c) cross-peaks for a mixture of four flavonoids in DMSO-d_6; experiment time 127 min

Angew Chem Int Ed **51**, 6460 (2012)

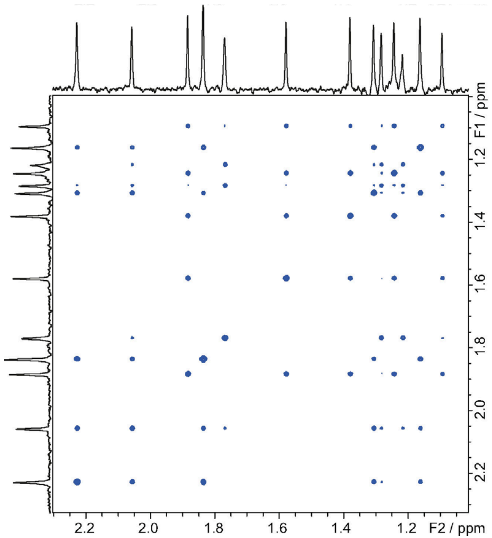
2D PSYCHE



TOCSY



F_1-PSYCHE-TOCSY



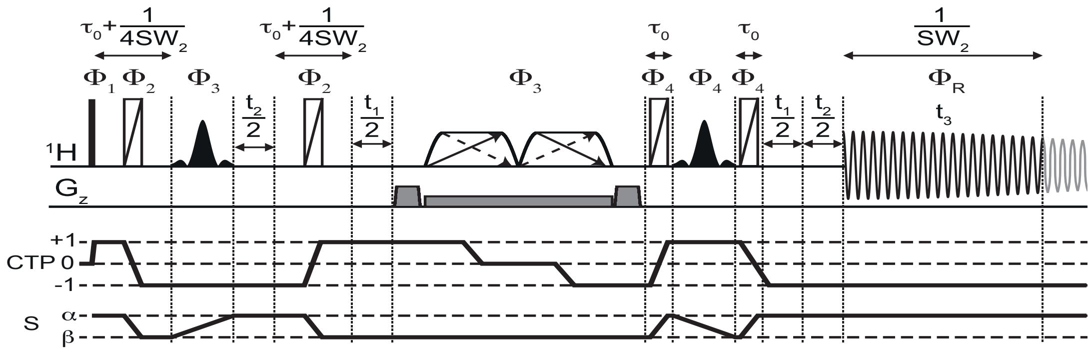
covariance-processed
F_1-PSYCHE-TOCSY

Using PSYCHE *J*-refocusing midway through t_1 in a TOCSY sequence then using covariance processing gives a completely decoupled estradiol TOCSY spectrum

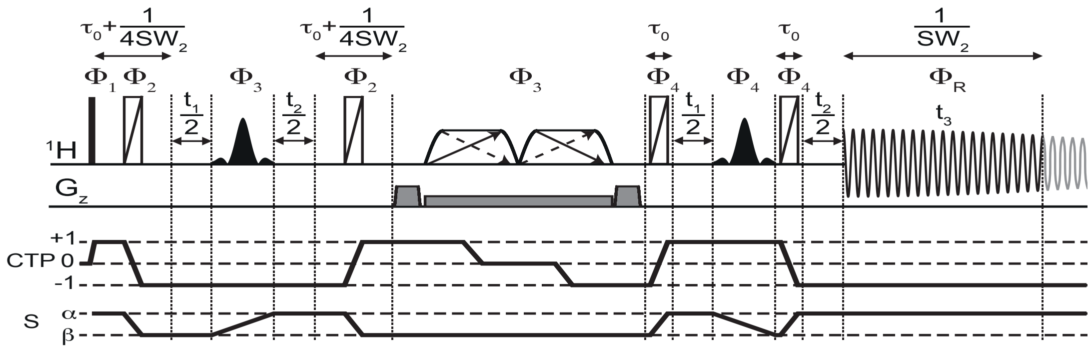
J Am Chem Soc **136**, 11867 (2014); *Chem Eur J*, in press; DOI: 10.1002/chem.201800524

Measuring Individual Couplings: PSYCHEDELIC

N-type modulation



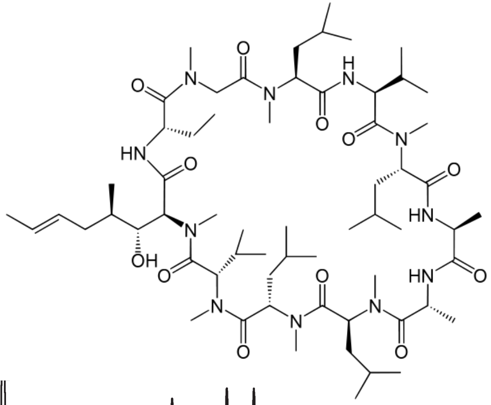
R-type modulation

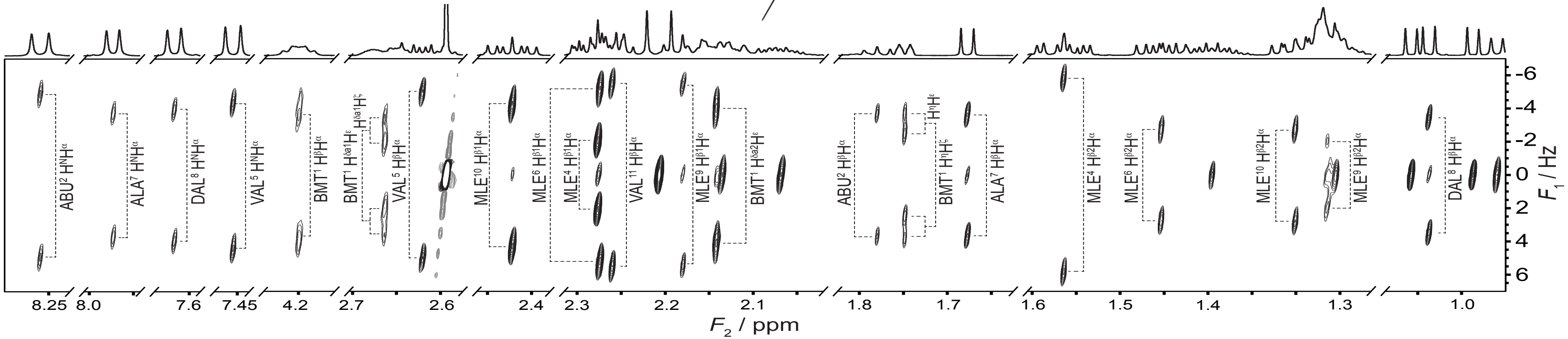


Pure Shift Yielded by Chirp Excitation to Deliver Individual Couplings: in a fully decoupled 2D*J* spectrum, selective pulses reintroduce just the couplings to a chosen spin or spins

Angew Chem Int Ed **55**, 1090 (2016)

PSYCHEDELIC spectrum of cyclosporin A





Applying the selective pulses to the H_α region allows all the couplings to this region to be measured without interference or overlap

Angew Chem Int Ed **55**, 1090 (2016)

Pure Shift NMR

The History: What ? Why ? Who ? When ? How ?

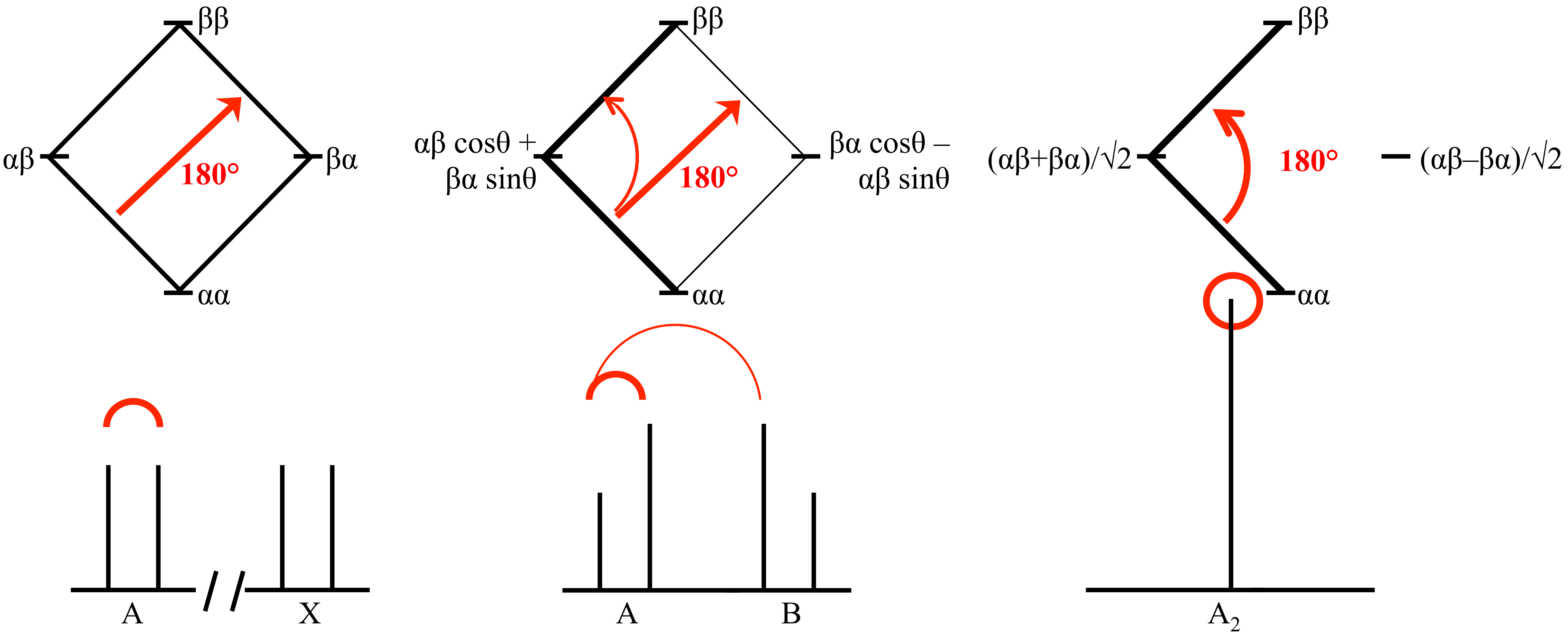
The Mechanics

Some Applications

Some Problems:

strong coupling, sidebands and artefacts

Strong coupling

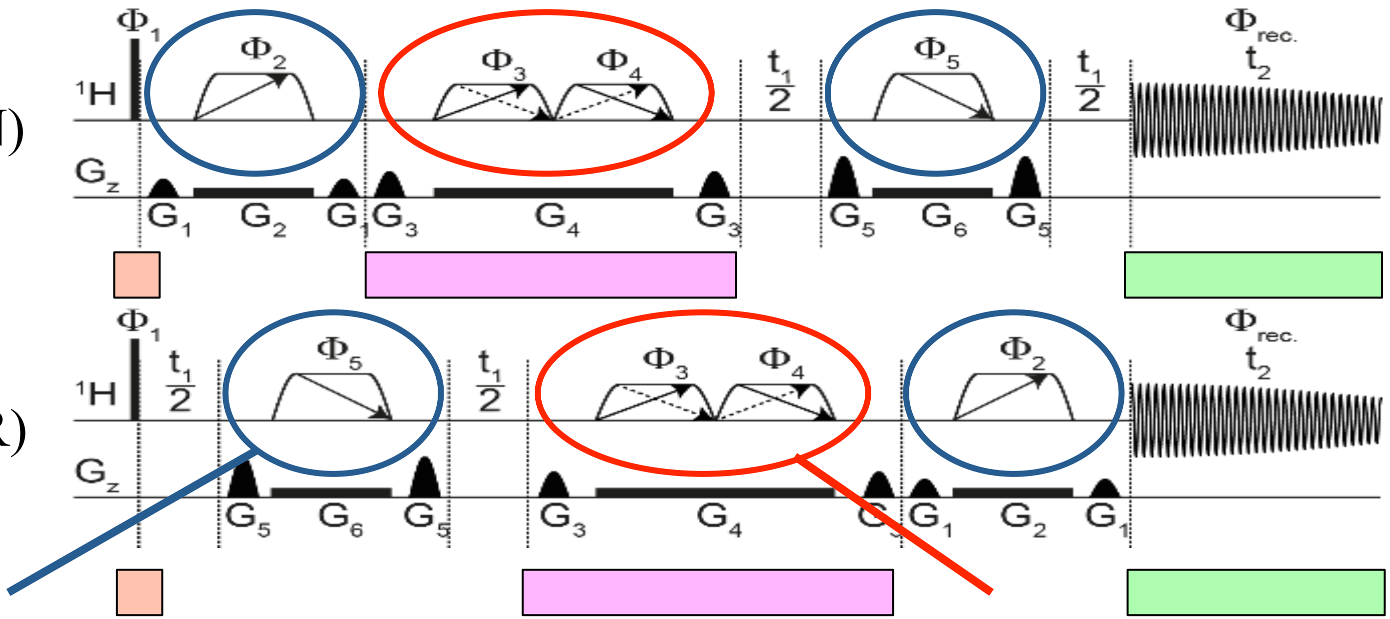


When $\Delta\delta$ is not large compared to J, transverse components of magnetization ($I_xS_x+I_yS_y$) interact as well as longitudinal (I_zS_z). This causes the spin states to mix, changing the relative strengths of coherences and opening up new coherence transfer pathways.

Triple Spin Echo PSYCHE 2D*J* Spectroscopy

Normal (N)

Reversed (R)

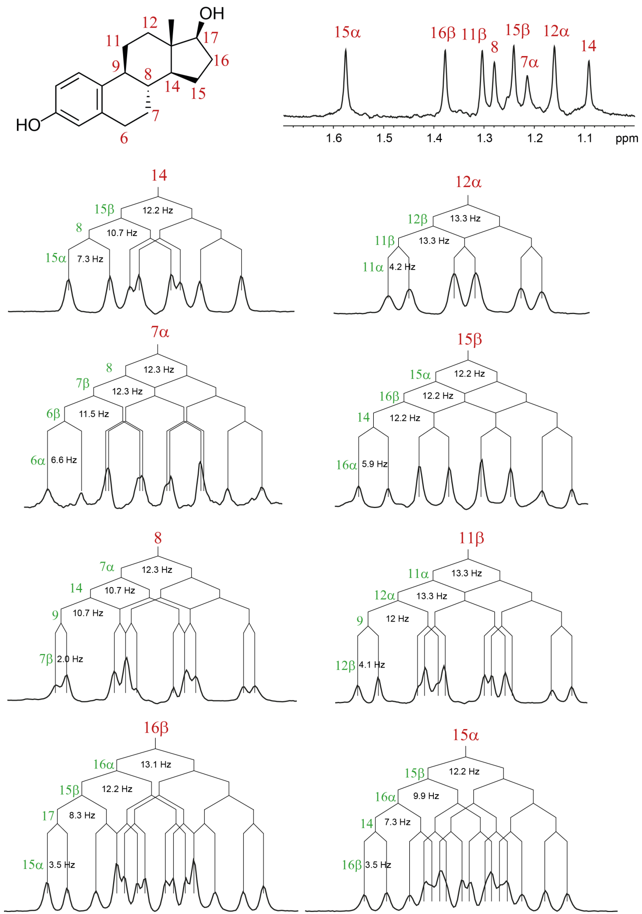


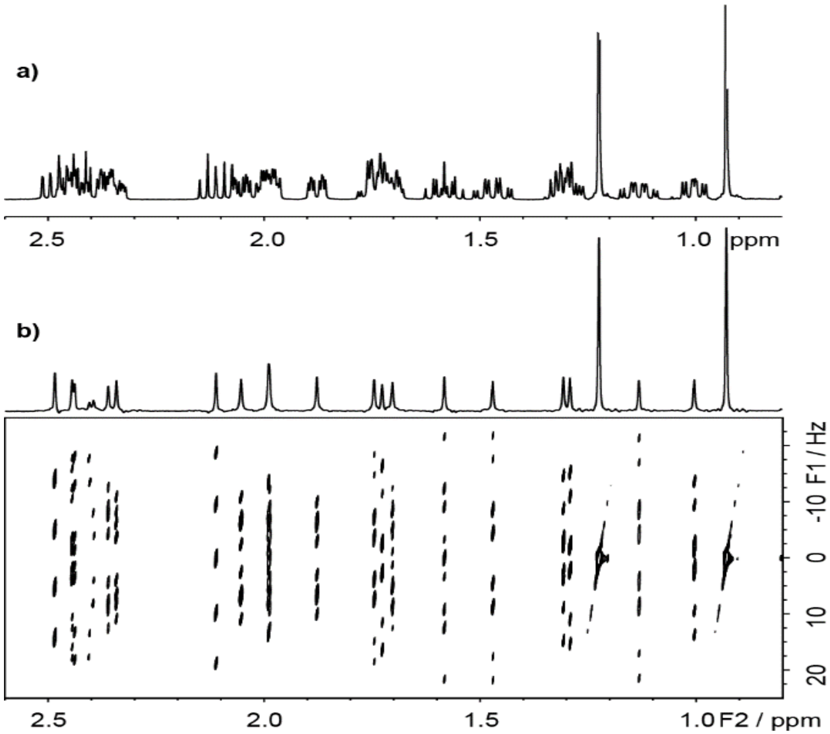
Chirp pulses generate echo at start of acquisition, but dephase signals that change shift, attenuating strong coupling artefacts

PSYCHE reverses sense of *J* evolution before (N) or after (R) evolution period, allowing echo/anti-echo processing

Chem Commun **51**, 15410 (2015)

Triple Spin Echo PSYCHE 2D*J* Spectroscopy



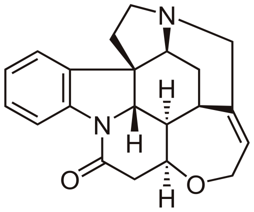


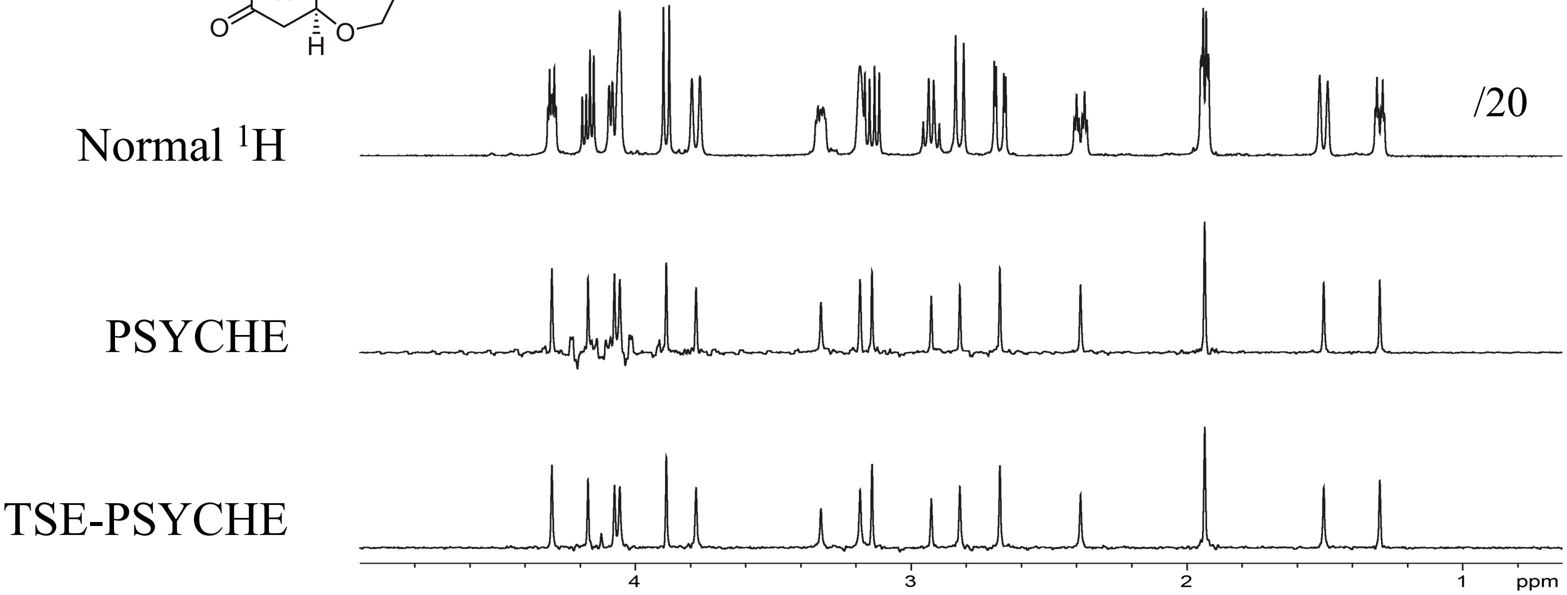
TSE-PSYCHE ^{1}H 2D*J* spectrum of estradiol

2D*J* spectrum is now in pure absorption mode, so 45° projection works fine

Chem Commun **51**, 15410 (2015)

1D TSE-PSYCHE: Tolerance of Strong Coupling

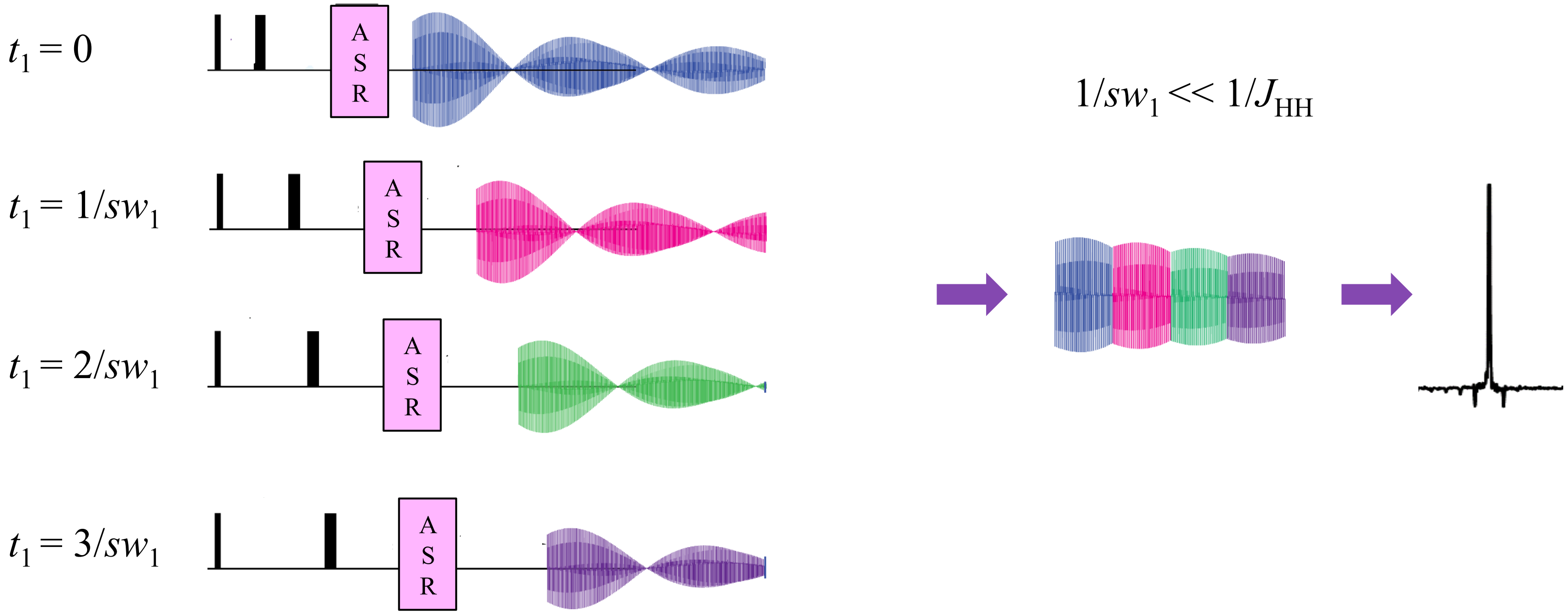




The TSE-PSYCHE 2D*J* sequence can also be used to measure 1D spectra

Chem Commun **51**, 15410 (2015)

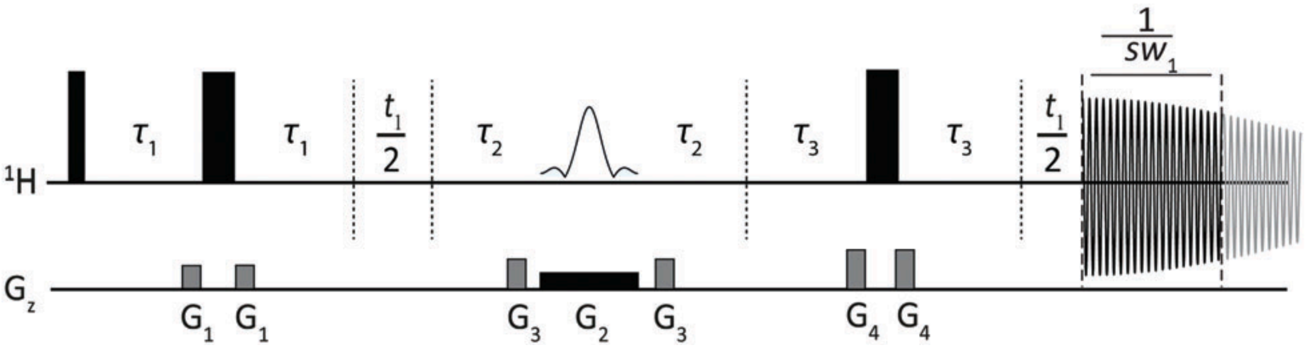
Constructing a Pure Shift FID: chunking sidebands

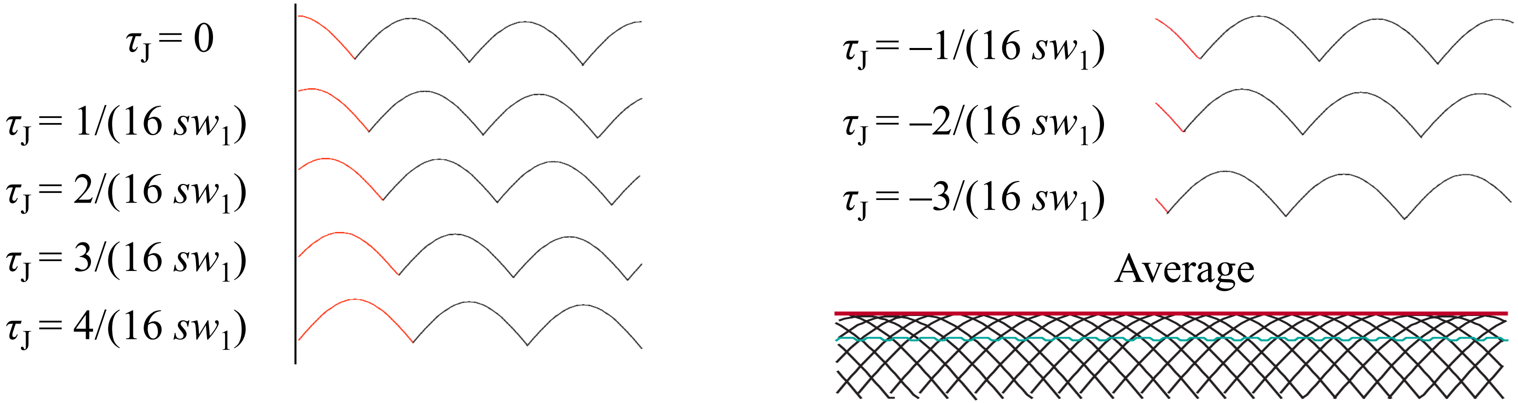


Successive experiments map out the decoupled signals. The residual effect of the couplings is to impose a small modulation of the assembled interferogram, with period $1/sw_1$, leading to small sidebands in the resulting spectrum with spacing sw_1.

J Magn Reson **271**, 99 (2016)

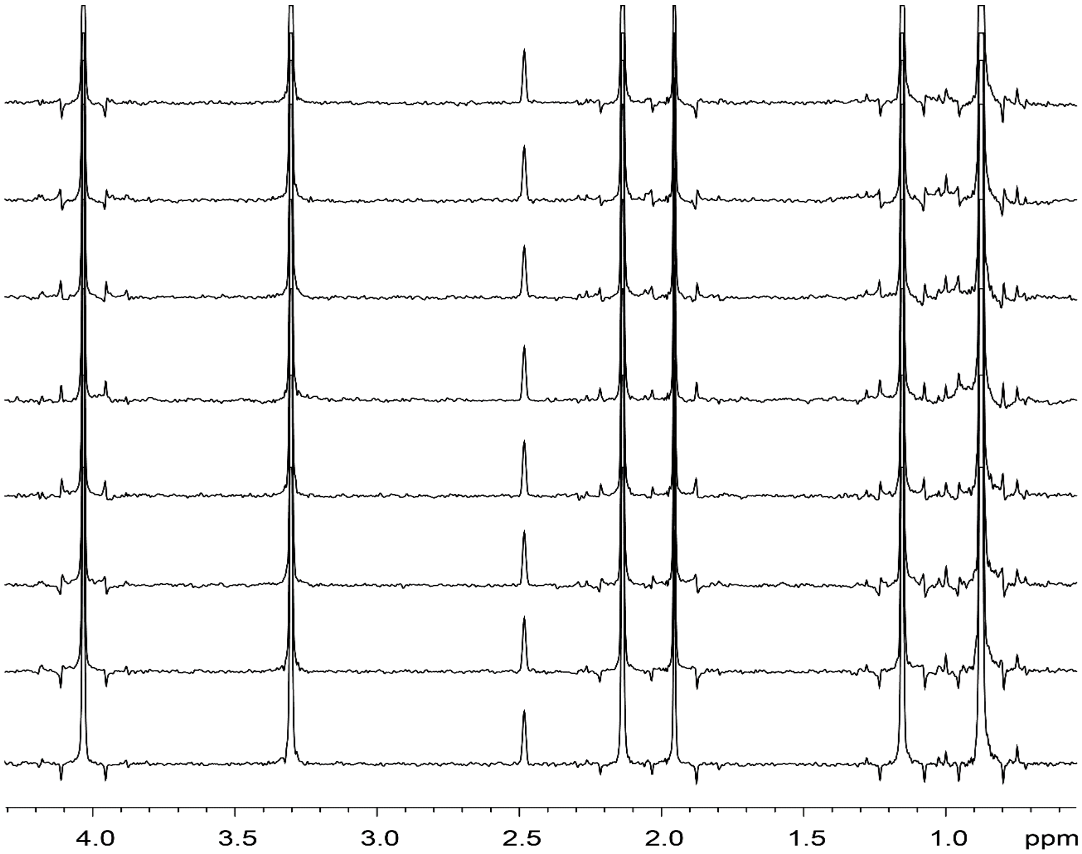
Changing the Residual *J* Modulation Phase: principle





Varying the net *J* evolution delay $\tau_J = 2(\tau_1 - \tau_3)$ changes the modulation phase, and hence the phases of the sideband signals. Averaging *N* different delays suppresses sidebands to order *N*–1.

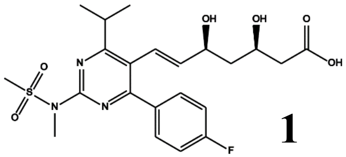
Changing the Residual J Modulation Phase: practice



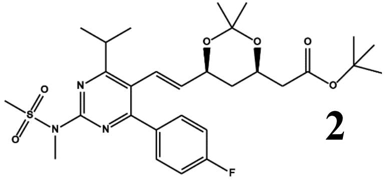
τ_J	modulation phase
$-3/(16\, sw_1)$	–135°
$-2/(16\, sw_1)$	–90°
$-1/(16\, sw_1)$	–45°
0	0
$1/(16\, sw_1)$	45°
$2/(16\, sw_1)$	90°
$3/(16\, sw_1)$	135°
$4/(16\, sw_1)$	180°

Sidebands go through 360*n*° phase shifts while the centreband signals are unchanged

Ultraclean Pure Shift NMR: SAPPHIRE sideband suppression



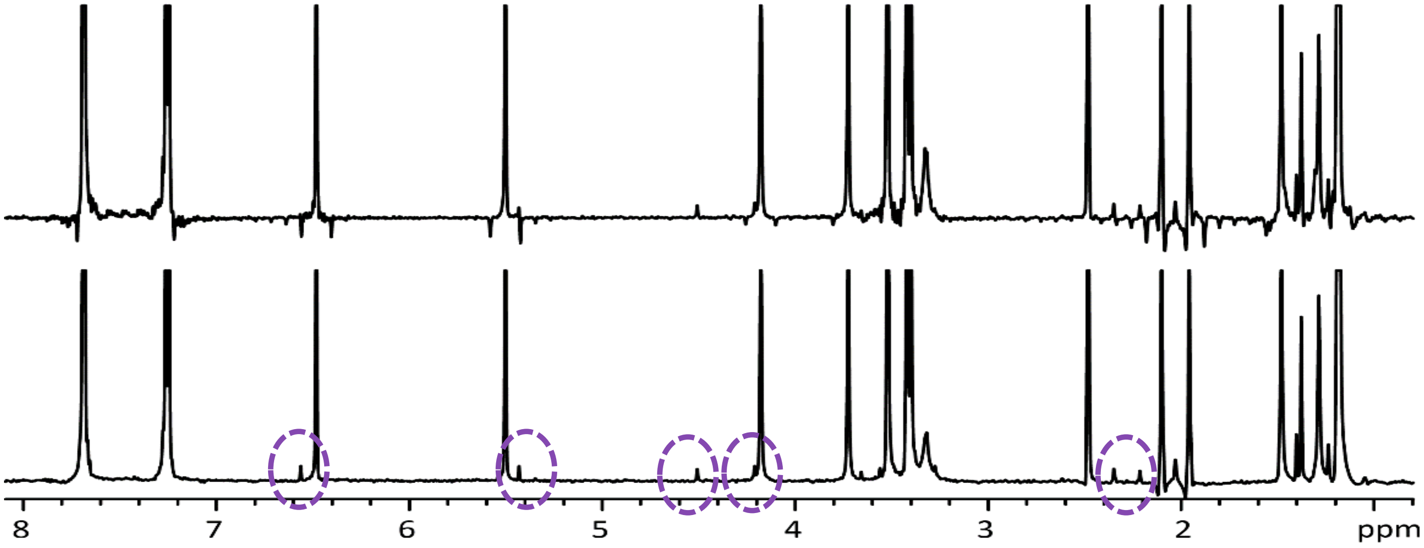
1



2

Normal ZS pure shift spectrum

ZS pure shift spectrum with sideband suppression

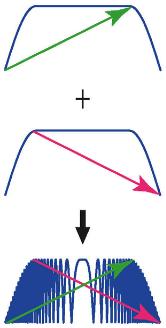


40 mM rosuvastatin (**1**) and 1 mM BEM (**2**) in DMSO-d_6

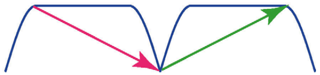
Sideband **A**veraging by **P**eriodic **PH**ase **I**ncrementation of **R**esidual ***J*** **E**volution allows reliable detection of signals of minor component signals

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

Improving PSYCHE: Saltire Pulses

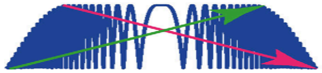


Adding two counter-sweeping chirp pulses gives a “saltire” pulse (named after the national flag of Scotland)



Normal PSYCHE “double *β*” sequence element

Double saltire element gives four times weaker recoupling artefacts



Single saltire element gives four times weaker recoupling artefacts and improves suppression of strong coupling and ZQC artefacts

Chem Eur J, in press; DOI: 10.1002/chem.201800524

Background Reading

"Pure Shift NMR Spectroscopy", R. W. Adams, in eMagRes, Wiley, 2014, DOI: 10.1002/9780470034590.emrstm1362

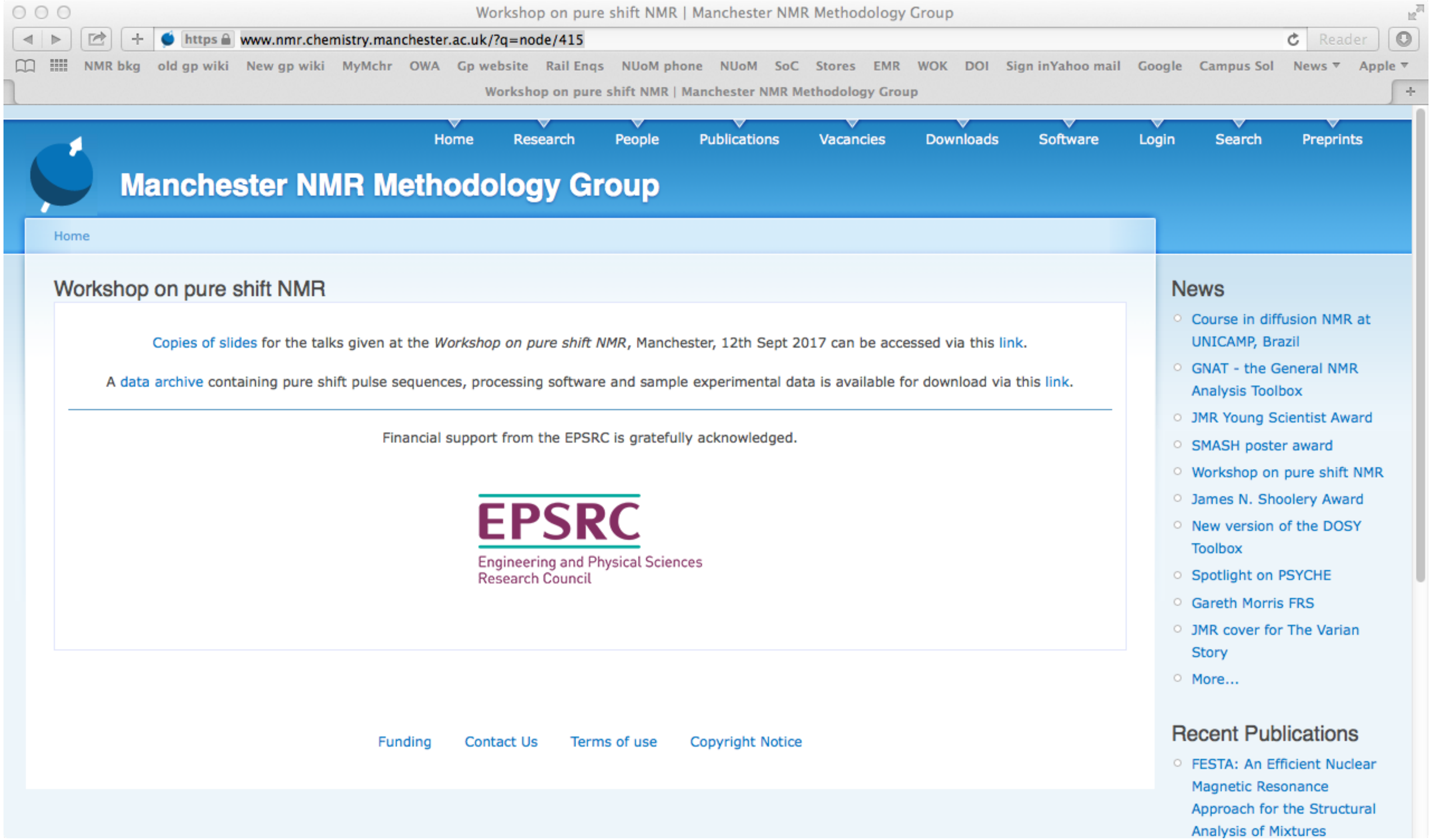
"Broadband ^{1}H homodecoupled NMR experiments: recent developments, methods and applications", L. Castañar, T. Parella, Magn. Reson. Chem. **53**, 399–426 (2015)

"Pure Shift NMR", K. Zangger, Prog. Nucl. Magn. Reson. Spectrosc. **86–87**, 1–20 (2015)

"Pure shift ^{1}H NMR: what is next?", L. Castañar, Magn. Reson. Chem. **57**, 47–53 (2017)

"PSYCHE Pure Shift NMR Spectroscopy", M. Foroozandeh, G.A. Morris and M. Nilsson, *Chem. Eur. J* in press, DOI: 10.1002/chem.201800524

https://www.nmr.chemistry.manchester.ac.uk/?q=node/415


Workshop on pure shift NMR | Manchester NMR Methodology Group

https www.nmr.chemistry.manchester.ac.uk/?q=node/415

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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.

Financial support from the EPSRC is gratefully acknowledged.

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News

- Course in diffusion NMR at UNICAMP, Brazil
- GNAT - the General NMR Analysis Toolbox
- JMR Young Scientist Award
- SMASH poster award
- Workshop on pure shift NMR
- James N. Shoolery Award
- New version of the DOSY Toolbox
- Spotlight on PSYCHE
- Gareth Morris FRS
- JMR cover for The Varian Story
- More...

Recent Publications

- FESTA: An Efficient Nuclear Magnetic Resonance Approach for the Structural Analysis of Mixtures

Acknowledgments

Ralph Adams, Juan Aguilar,

Laura Castañar, Yingxian Chen,

Jonathan Clayden, Guilherme Dal Poggetto,

Mohammadali Foroozandeh, Peter Kiraly,

Pinelopi Moutzouri, Mathias Nilsson,

Liladhar Paudel

Manchester

Steve Coombes, Andy Phillips — AstraZeneca

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