DEPARTMENT
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Polishing GEMSTONEs

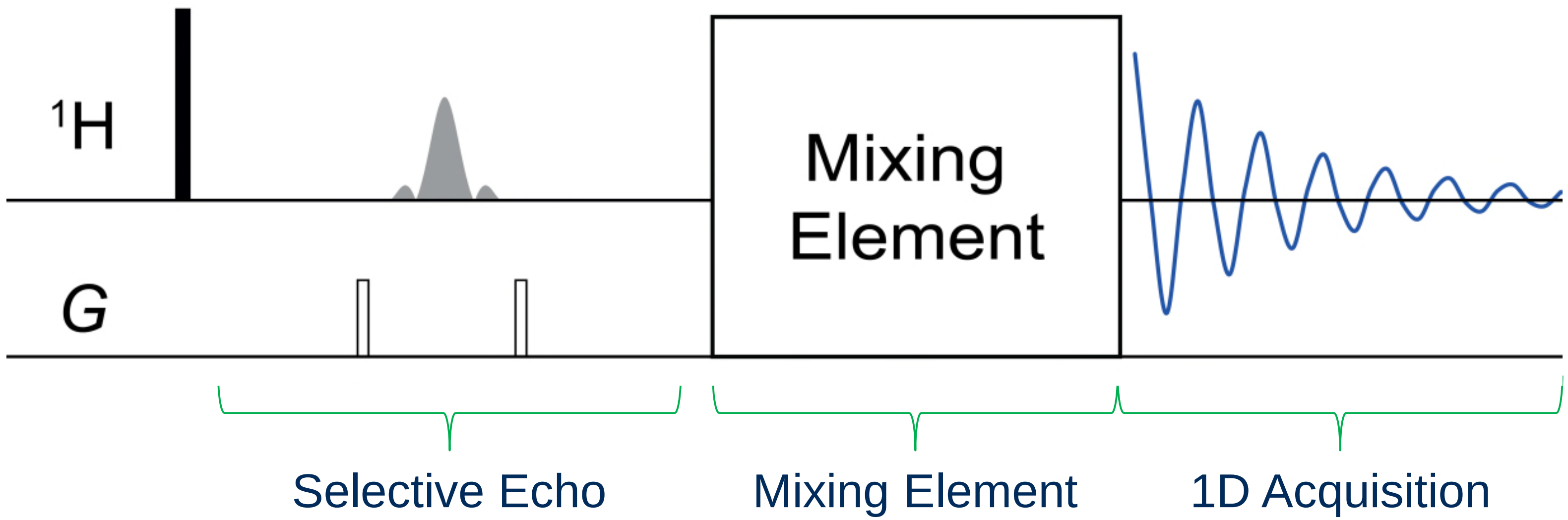
Optimisation of Ultra-Selective NMR Experiments

22/06/2023

Overview

- Conventional selective experiments & CSSF
- Gradient enhanced selective excitation (i.e GEMSTONE)
- Road to success – implementation (Bruker systems)

Selective NMR Experiments



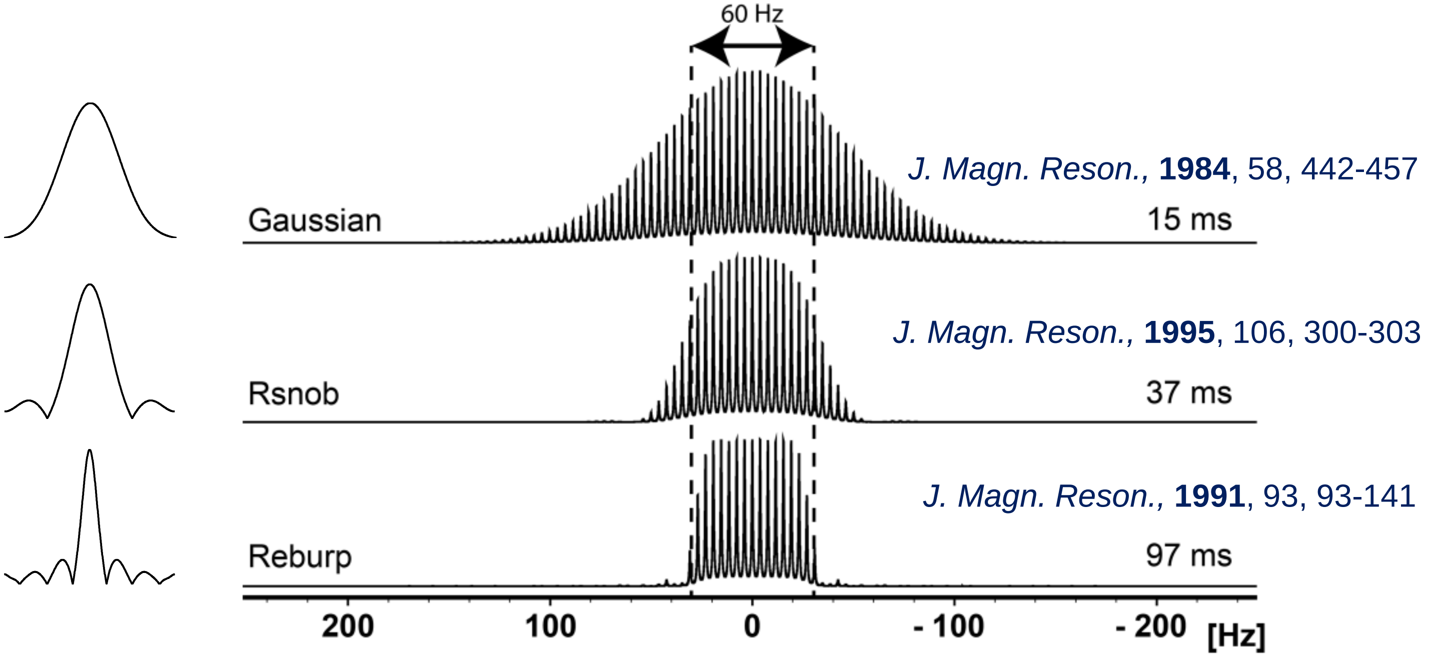
✓ Shorter experiments

✓ Targeted analyses

✓ Simplified spectra

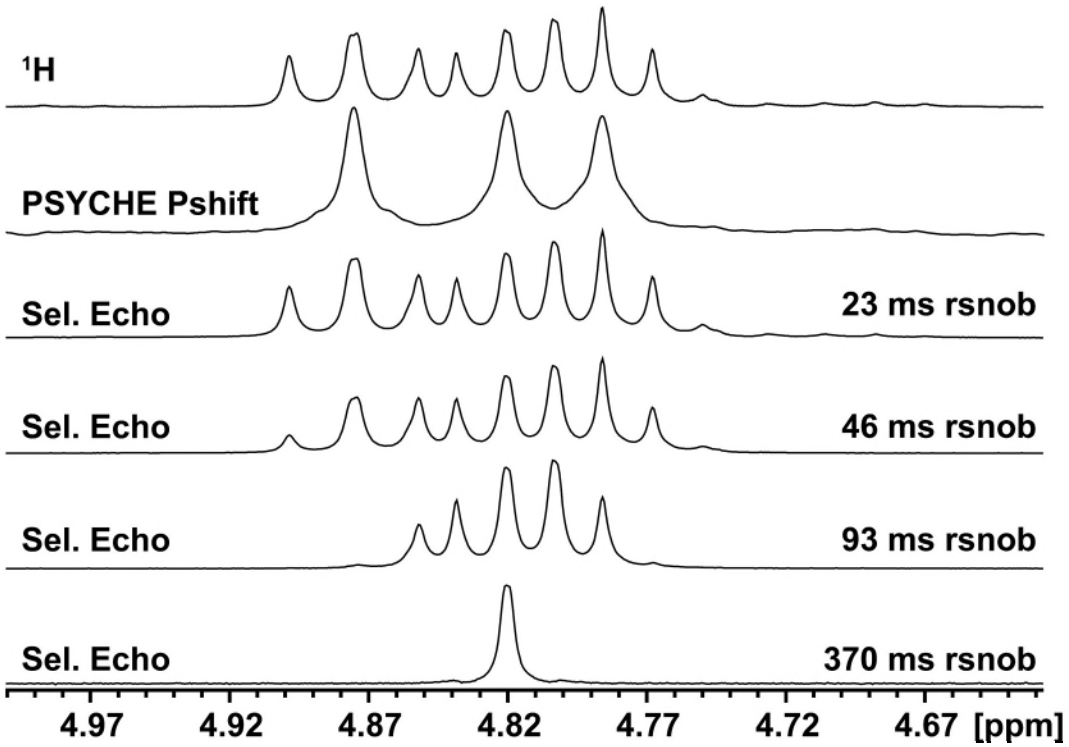
Selective Pulses – Refocusing Profiles

Shapes of pulses determine the refocusing profiles

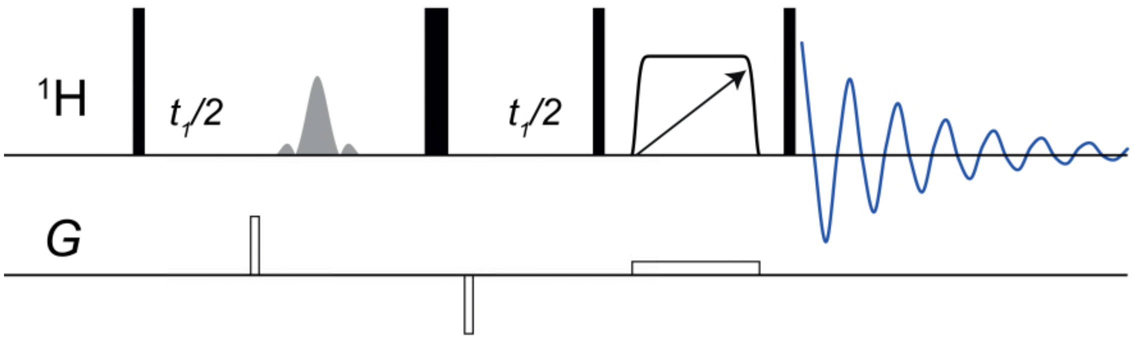


Cannot discriminate between overlapping multiplets !!!

Selective Pulses – Multiplet Overlap



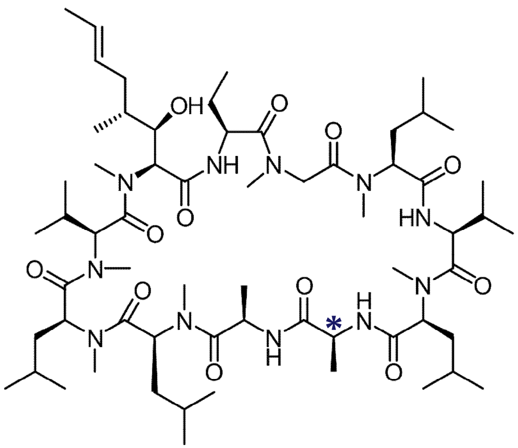
Chemical Shift Selective Filter

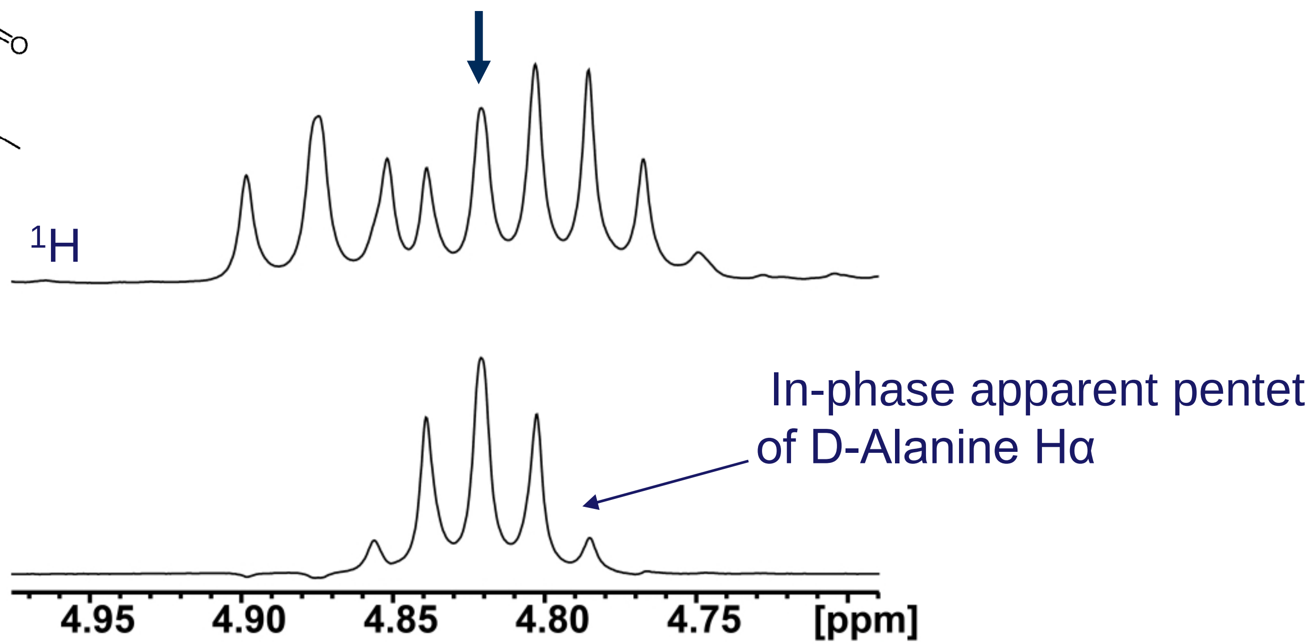


Pseudo 2D experiment

- Increment an evolution delay
- Off resonance spins develop a phase as a function of t_1
  - Average to zero by summing FIDs in 2D array

CSSF – Cyclosporin Example





Ultrafast CSSF

Gradient ***E***nhanced ***M***ultiplet ***S***elective ***T***argeted ***O***bservation ***N***MR ***E***xperiments

Uses similar ideas to CSSF

Achieves ultra-selective observation in a single scan

GEMSTONE Family

UNIVERSITY OF OXFORD

GDCh Communications Angewandte Chemie International Edition

NMR Spectroscopy Hot Paper

How to cite: *Angew. Chem. Int. Ed.* **2021**, *60*, 666–669
International Edition: doi.org/10.1002/anie.202011642
German Edition: doi.org/10.1002/ange.202011642

Single-Scan Selective Excitation of Individual NMR Signals in Overlapping Multiplets

Peter Kiraly, Nicolas Kern, Mateusz P. Plesniak, Mathias Nilsson, David J. Procter, Gareth A. Morris, and Ralph W. Adams**

ChemComm ROYAL SOCIETY OF CHEMISTRY

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Cite this: *Chem. Commun.*, 2021, **57**, 2368

Received 10th December 2020, Accepted 26th January 2021

Single-scan ultra-selective 1D total correlation spectroscopy†

Peter Kiraly, ‡ Mathias Nilsson, Gareth A. Morris and Ralph W. Adams *

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Cite this: *Chem. Commun.*, 2023, 59, 5854

Received 6th February 2023, Accepted 29th March 2023

Ultra-selective, ultra-clean 1D rotating-frame Overhauser effect spectroscopy†

Emma L. Gates, [a] Marshall J. Smith, [a] Jonathan P. Bradley, [b] Myron Johnson, [b] Göran Widmalm, [c] Mathias Nilsson, [a] Gareth A. Morris, [a] Ralph W. Adams *[a] and Laura Castañar *[ad]

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Cite this: *Chem. Commun.*, 2023, 59, 6734

Received 17th March 2023, Accepted 19th April 2023

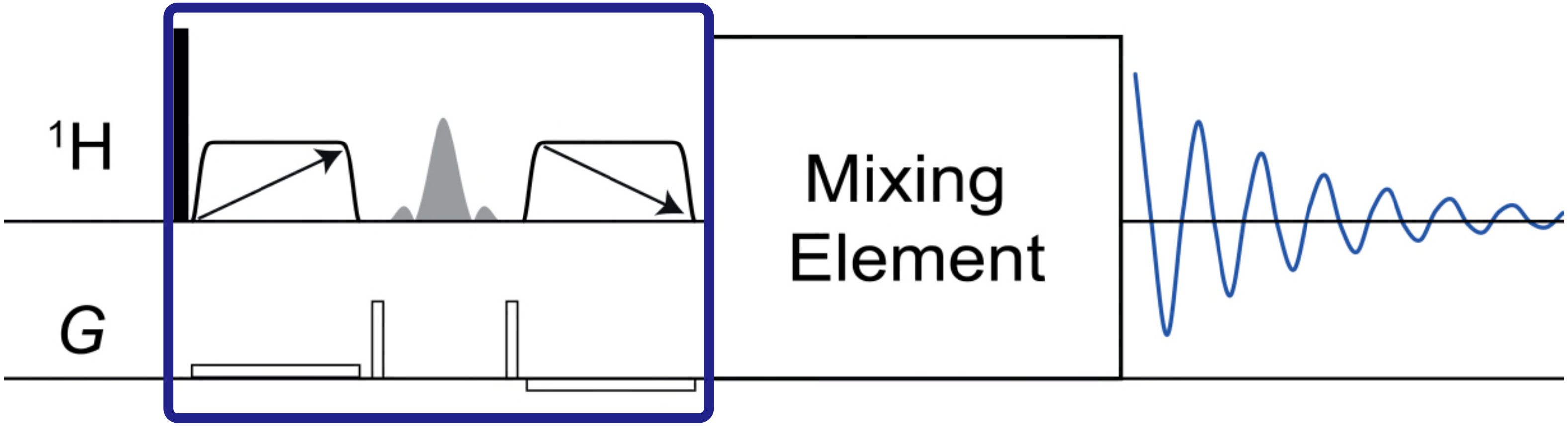
Ultra-selective 1D clean in-phase correlation spectroscopy†

Daniel A. Taylor, [a] Peter Kiraly, [b] Paul Bowyer, [c] Mathias Nilsson, [a] Laura Castañar, [ad] Gareth A. Morris [a] and Ralph W. Adams *[a]

More to come…

GEMSTONE Experiments

Initial selective element replaced with GEMSTONE element

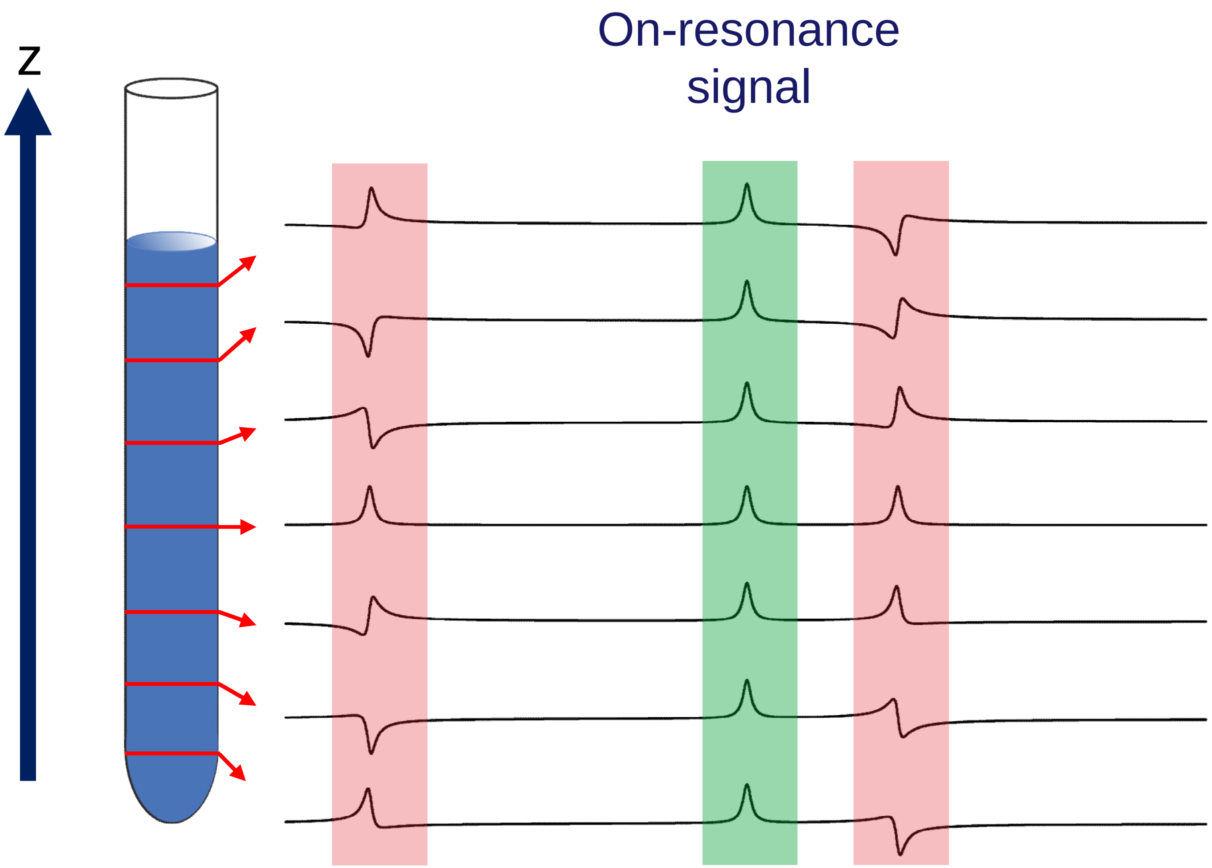


Want to observe individual multiplets

Want to observe in phase multiplets with absorption mode line shapes

Want J_{HH} evolution to be refocused

Aim Of The Game

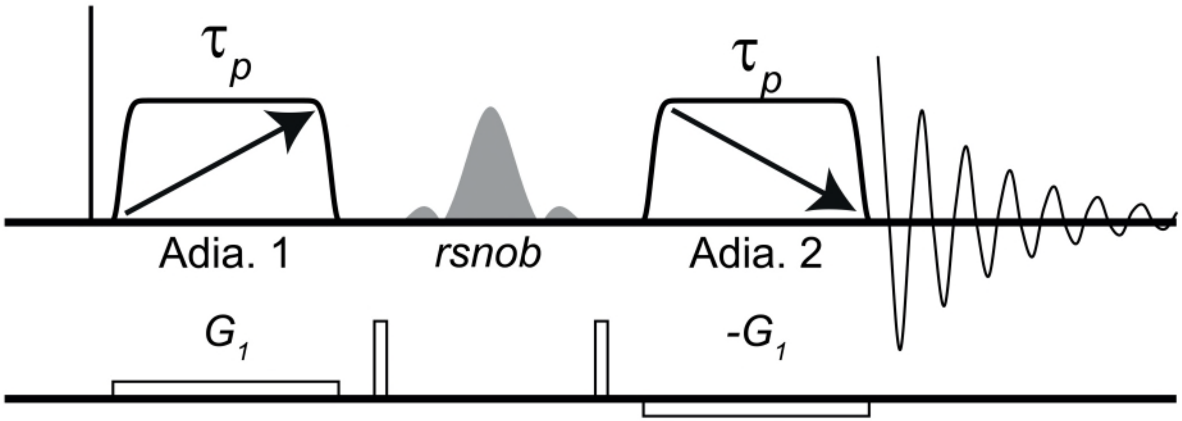


- **On-resonance** signal retains the same phase throughout the NMR tube
- **Off-resonance** signals acquire a spatially-dependent phase

⇨ Off-resonance signals average to zero over the length of the NMR tube

SPINACH simulations – many thanks to Emma Gates

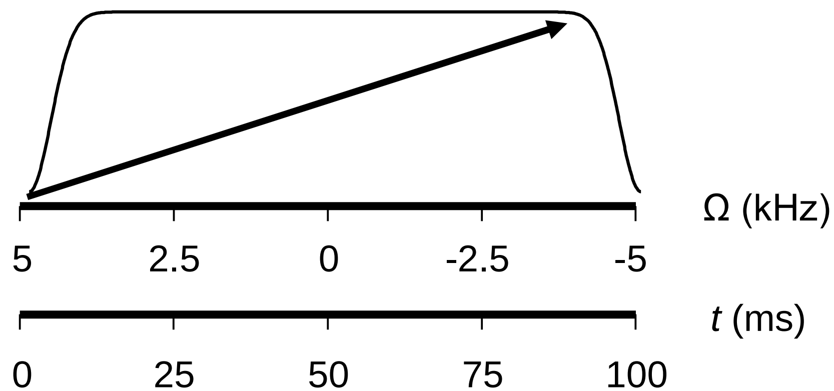
GEMSTONE Pulse Sequence



- Semi-selective *rsnob*
  - Refocuses J_{HH}
  - Suppression of signals far from resonance
  - Inversion of active spins between adiabatic pulses
- Adiabatic pulses and pulsed field gradient
  - Spatial encoding of signals – samples a continuous array of t_1 evolution times

Frequency Swept Pulses

Maps frequency onto time



Pulse linearly sweeps from +BW/2 to –BW/2 for the duration of the pulse

Inversion of a spin depends on its frequency from resonance

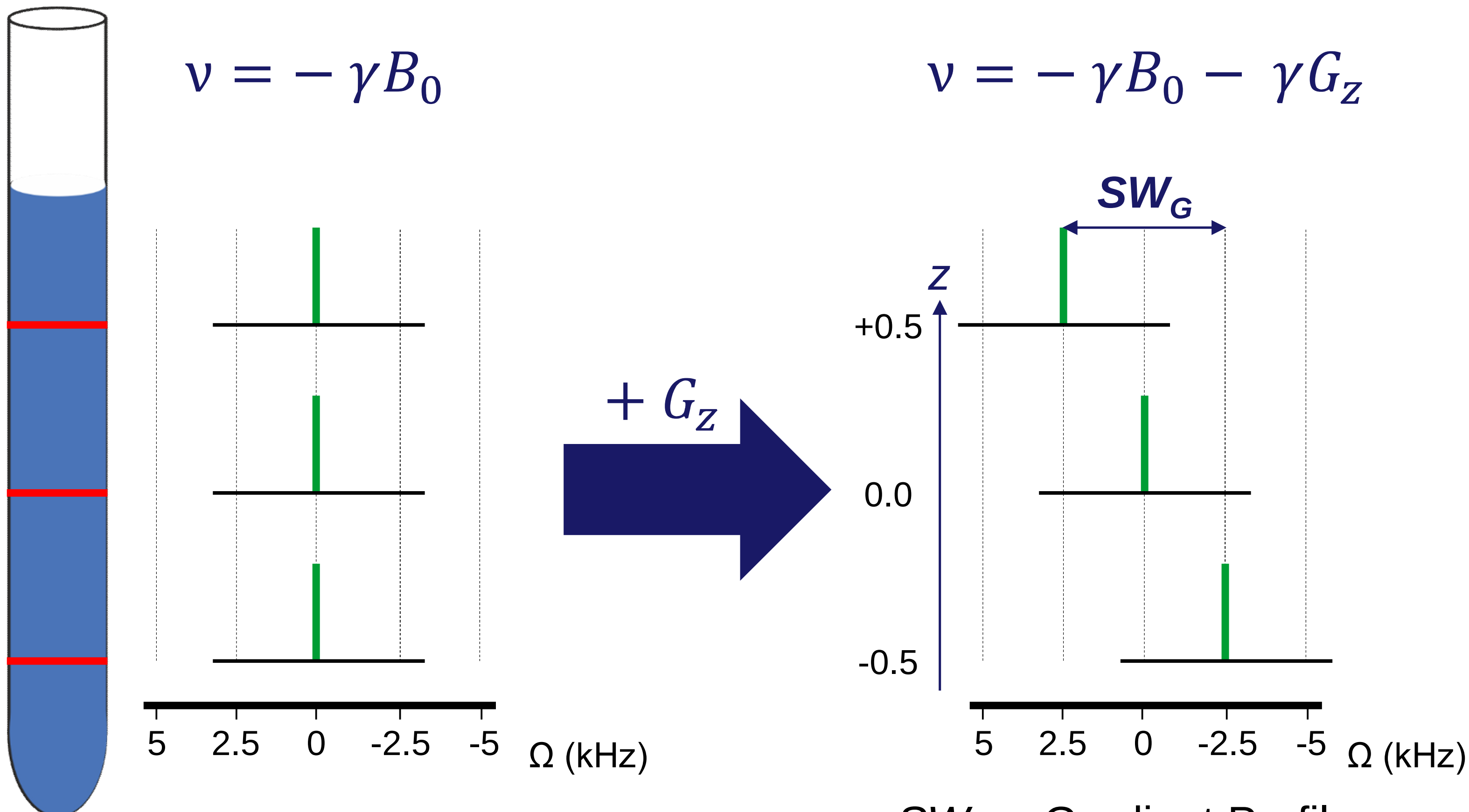
Assuming the instantaneous flip approximation

Gradient Profile

Frequency of spins becomes spatially dependent during a gradient

$$\nu = -\gamma B_0$$

$$\nu = -\gamma B_0 - \gamma G_z$$



SW_G = Gradient Profile

Gradient Profile

Frequency of spins becomes spatially dependent during a gradient

$$\nu = -\gamma B_0$$

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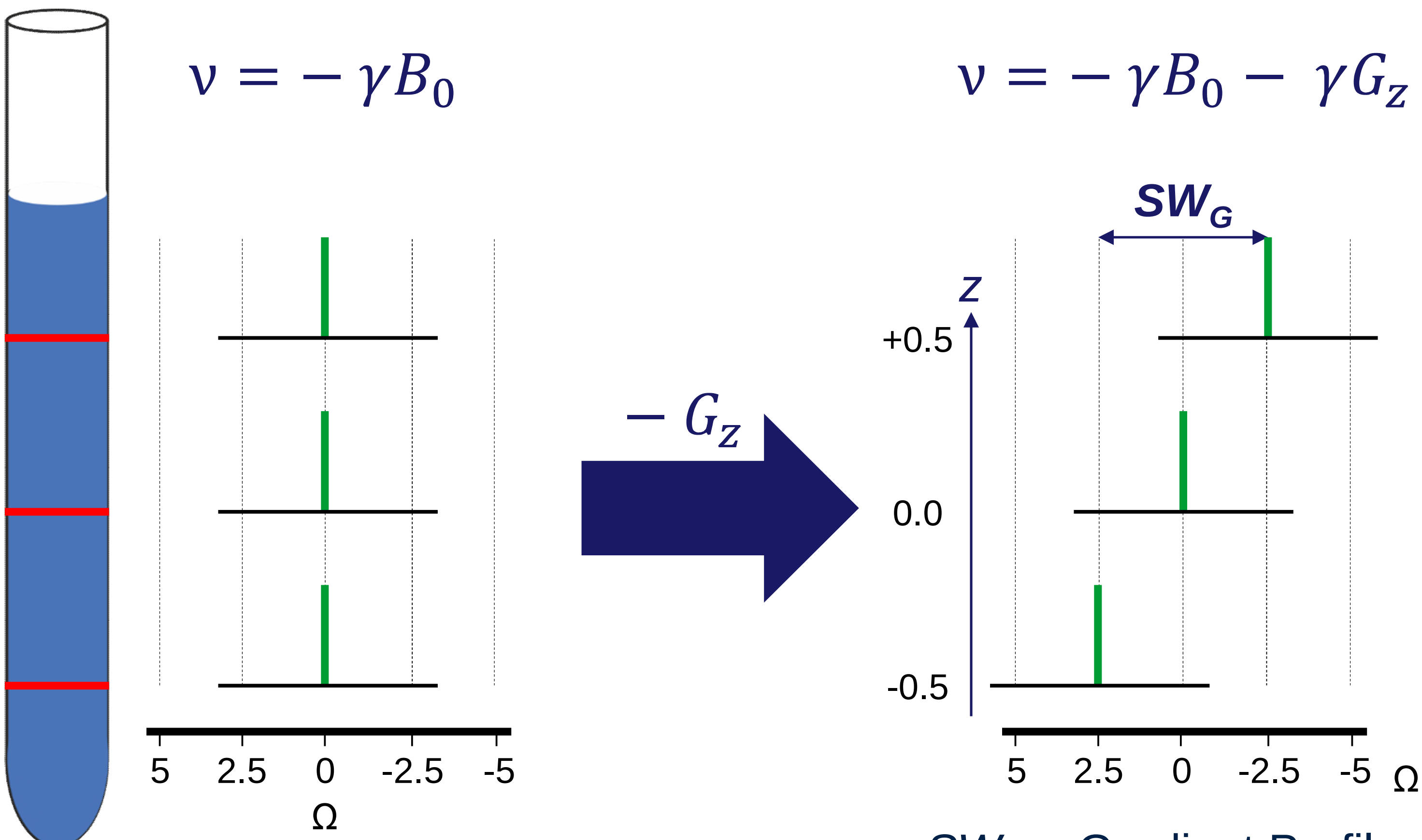
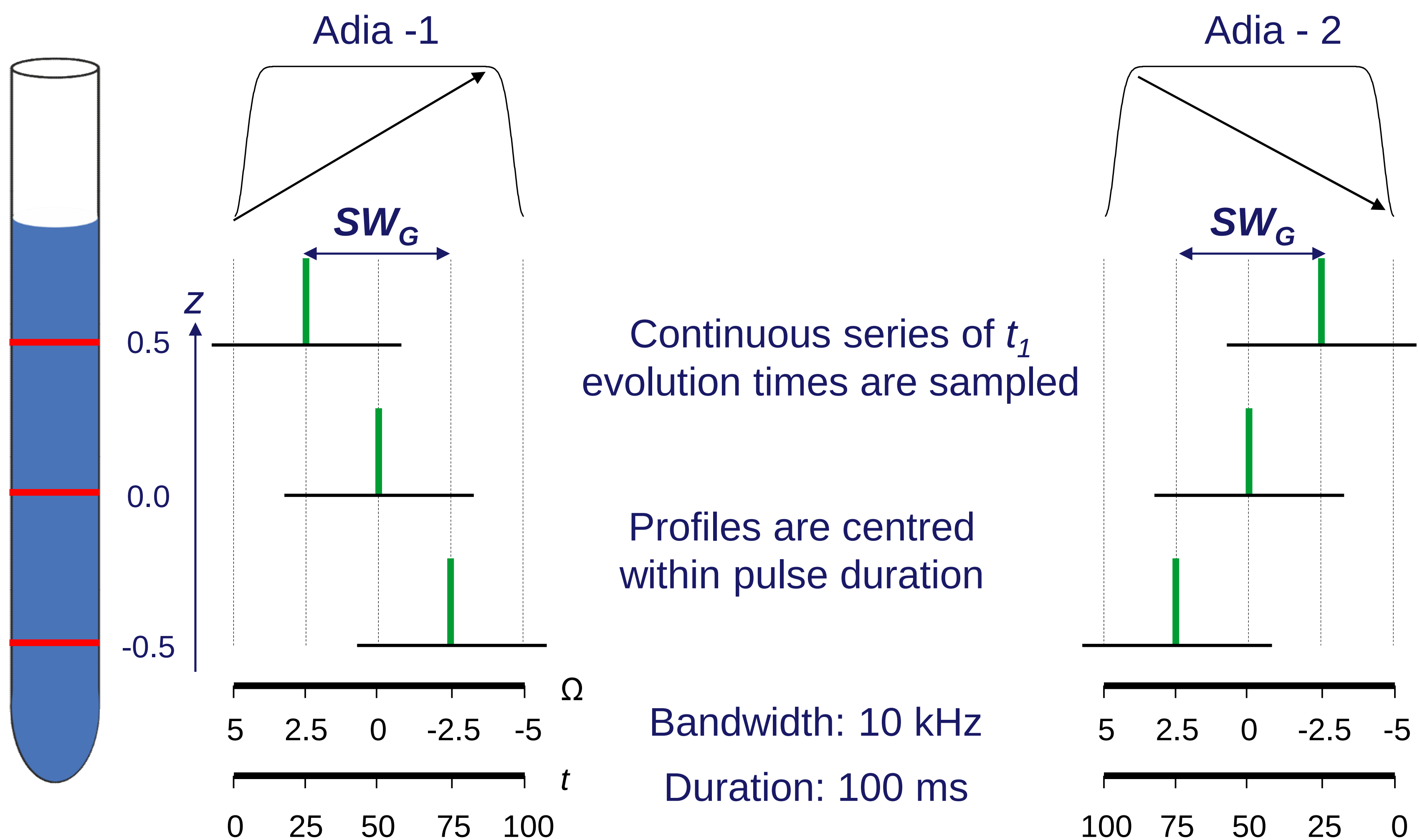


SW_G = Gradient Profile

Spatio-Temporal Averaging – on resonance spins



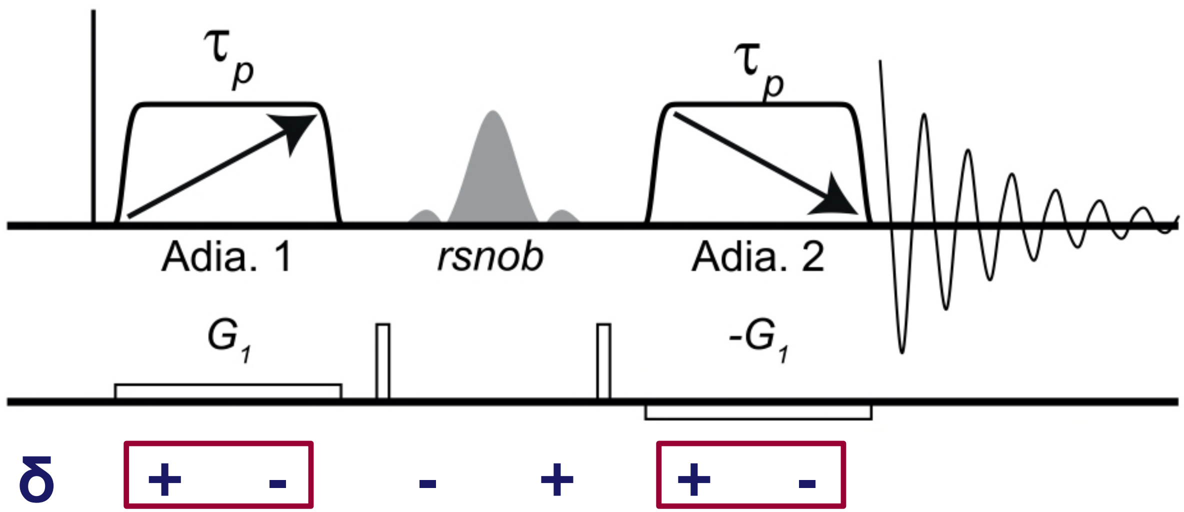
Continuous series of t_1 evolution times are sampled

Profiles are centred within pulse duration

Bandwidth: 10 kHz

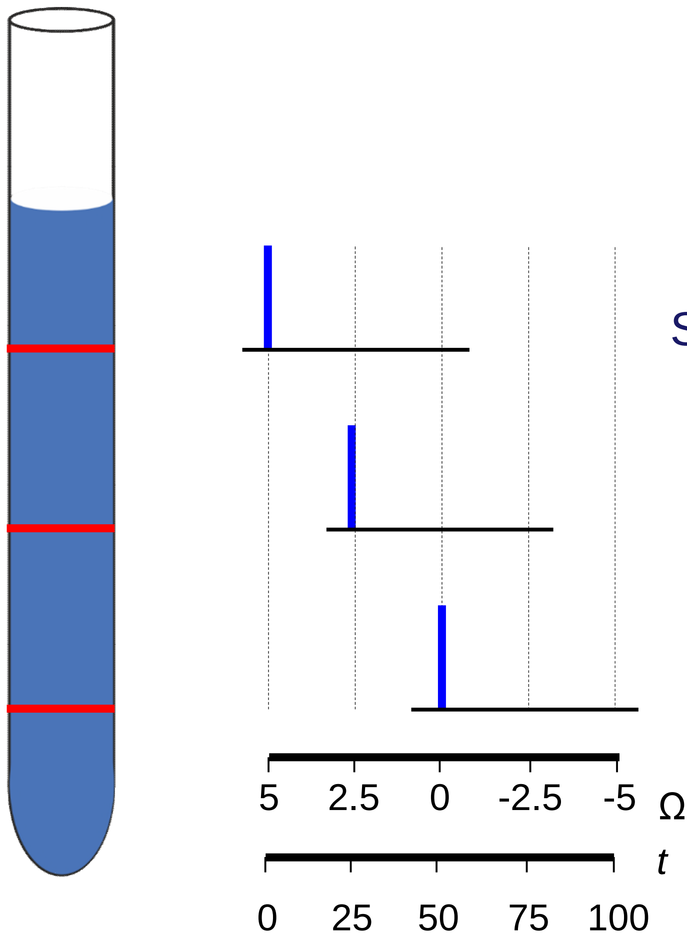
Duration: 100 ms

Desired Spin Evolution – Chemical Shifts



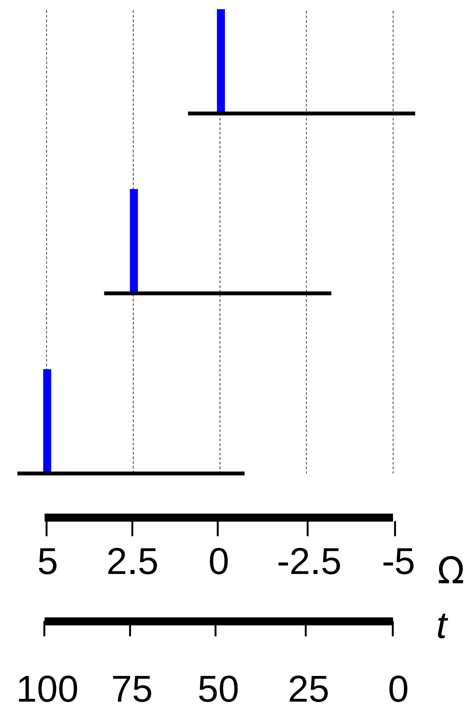
Same sense of chemical shift evolution entering both adiabatic pulses

Spatio temporal averaging – off resonance spins

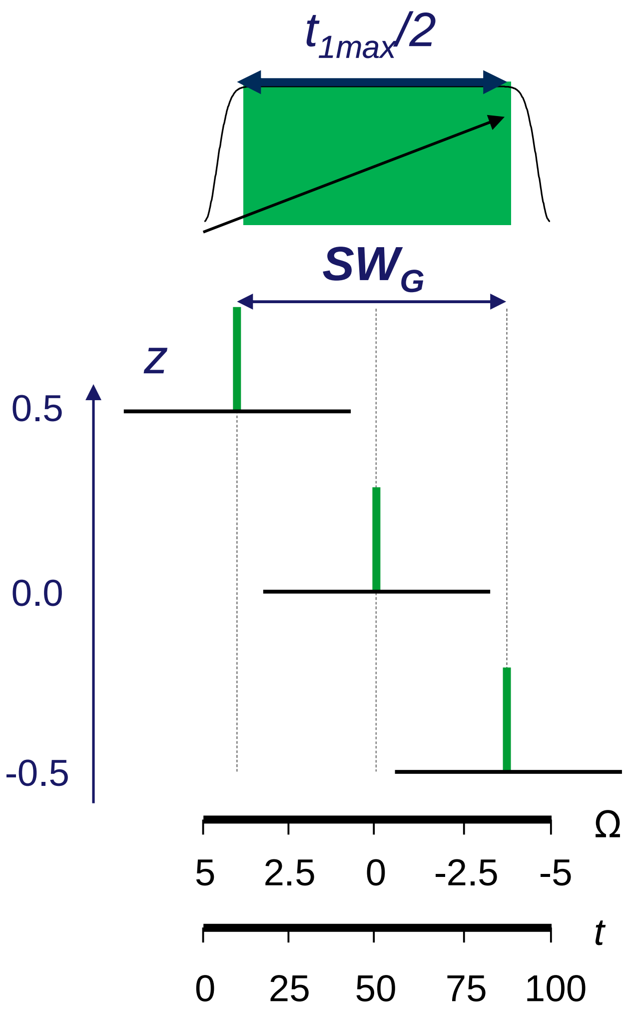


Spin profiles not centred on pulse duration

Spin evolution is spatially dependent !!!



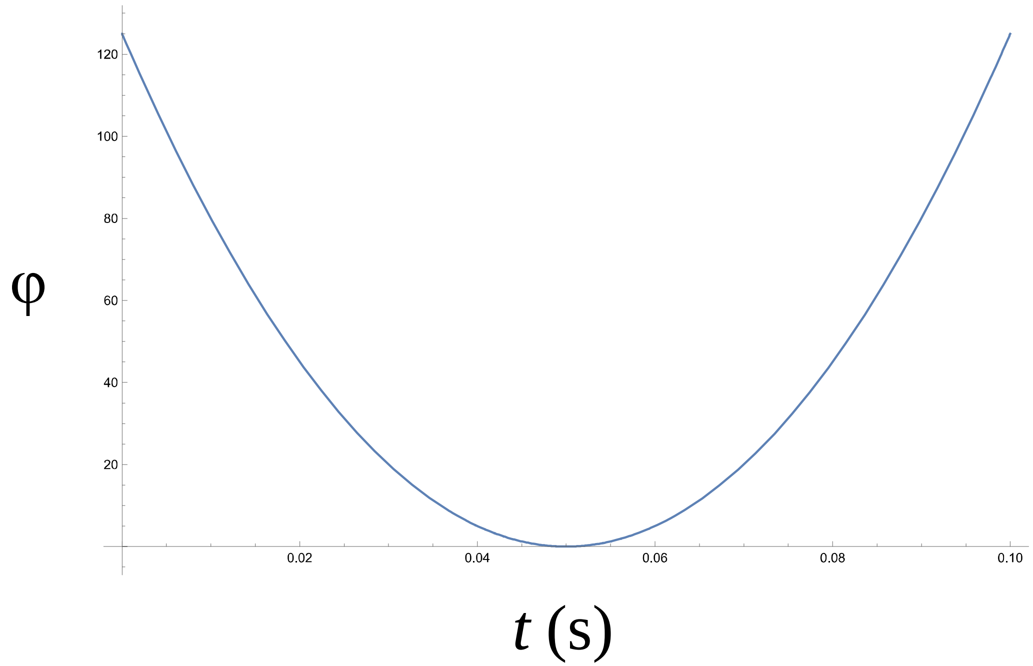
Selectivity determined by t_{1max}



Longer t_{1max} → greater selectivity

Matching SW_G to full bandwidth wastes magnetisation – smooth regions of the pulse imperfectly flip spins

Pulse Phase Modulation

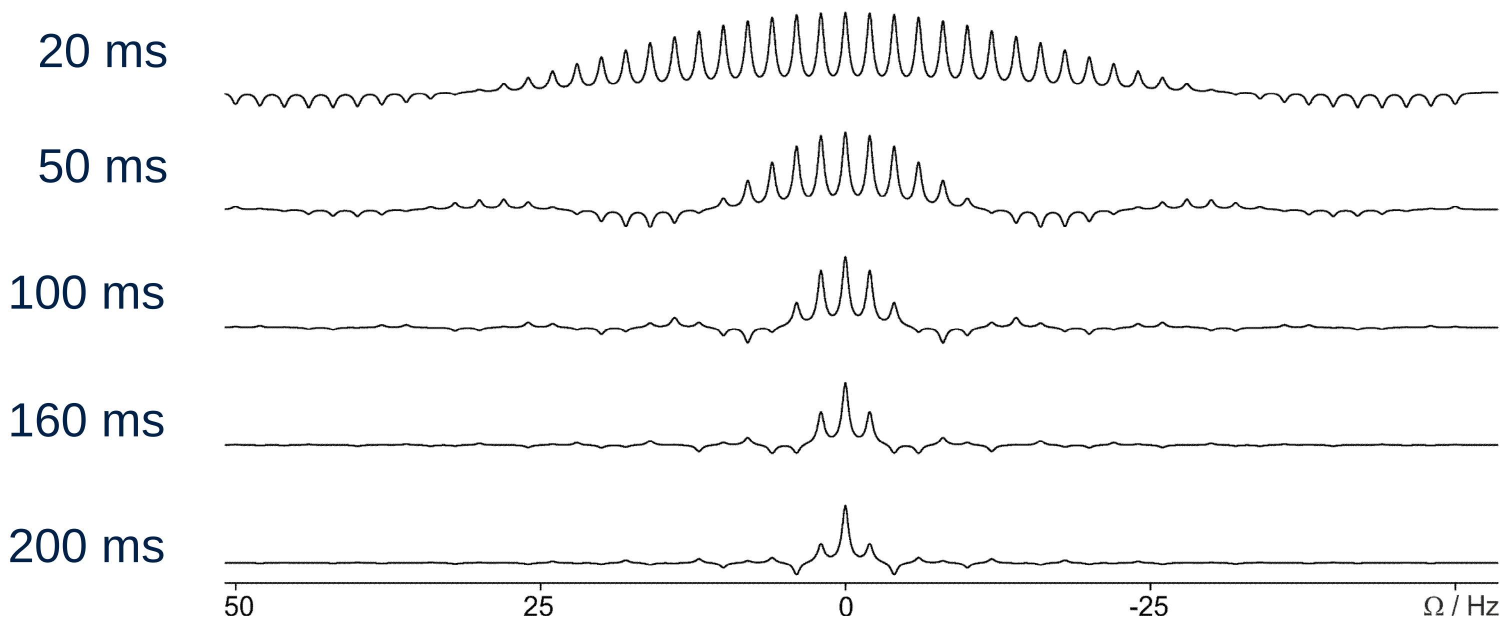


Imparts a time dependent phase shift on spins

Excitation Profile

Recommended pulse durations: 70-150 ms

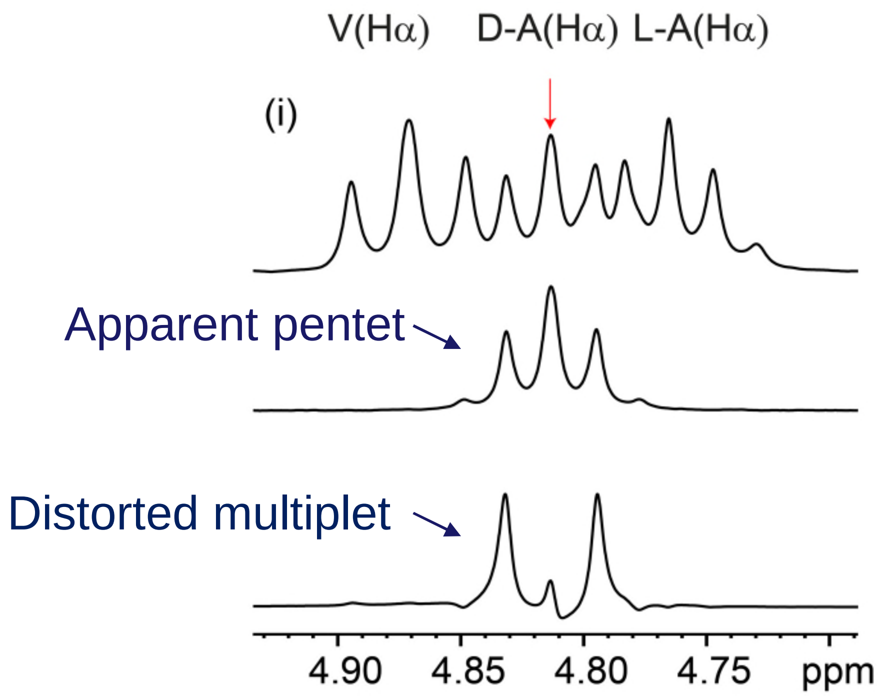
Excitation profile takes the form of a sinc function



Width of central lobe dependent on t_{1max} (set by adiabatic pulse duration)

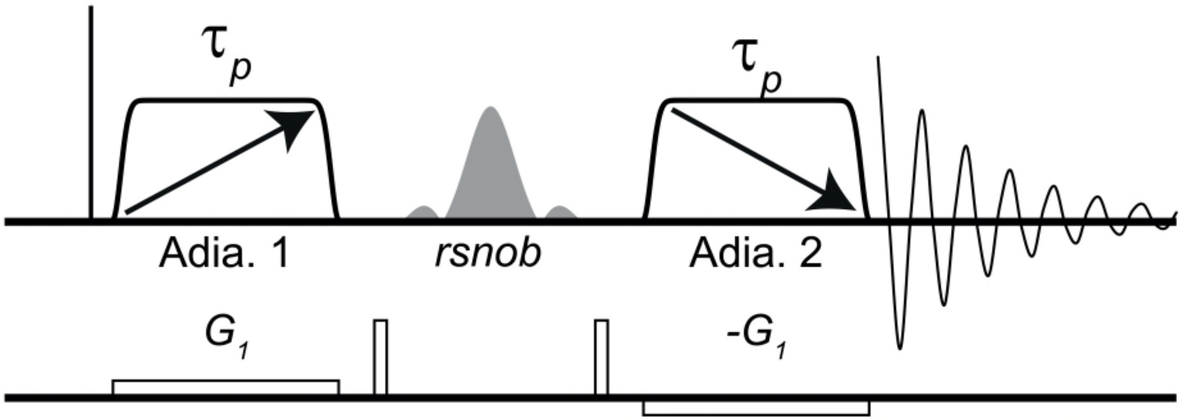
Observation of an Individual Multiplet – Cyclosporin Example

Targeting H_α of D-Alanine



Region from Cyclosporin A spectrum

Scalar coupling evolution

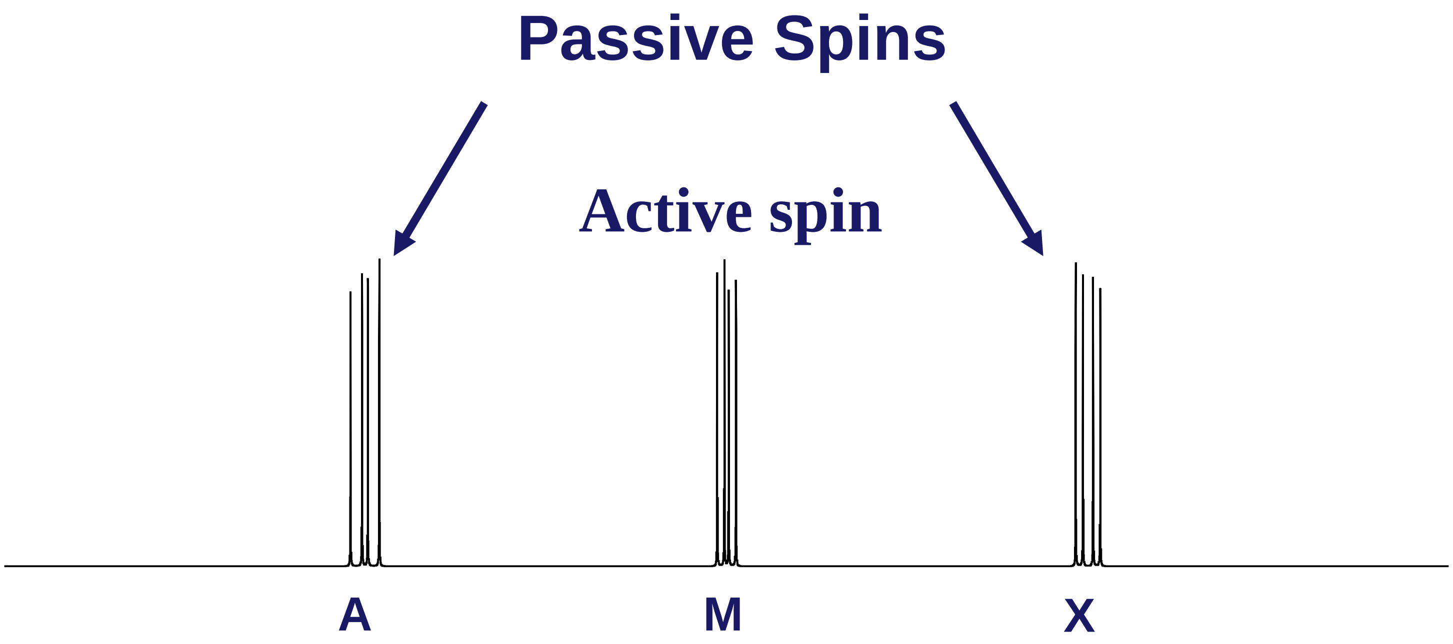


J_{HH} + + + - - -

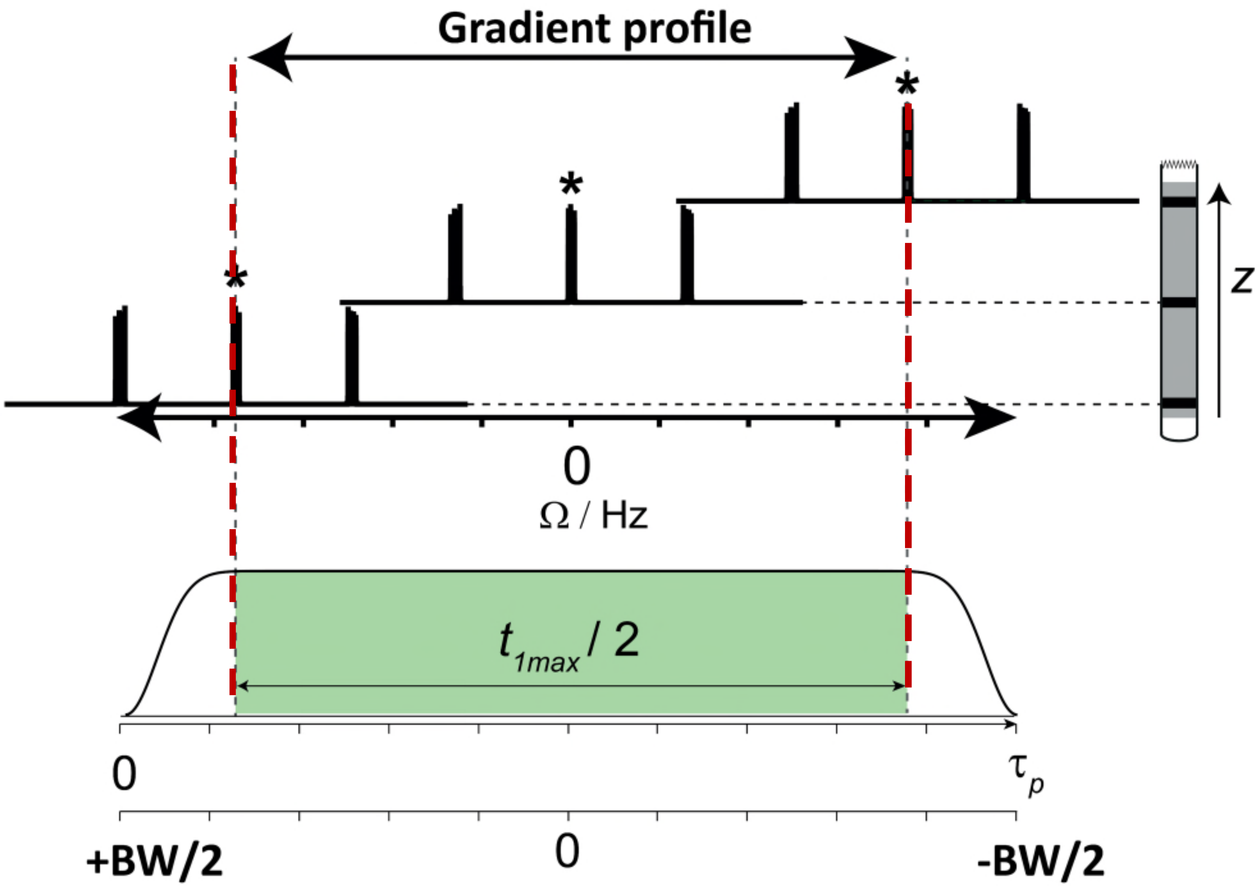
J_{HH} becomes refocused at the beginning of acquisition in perfect scenario

Practically, a compromise needs to be made

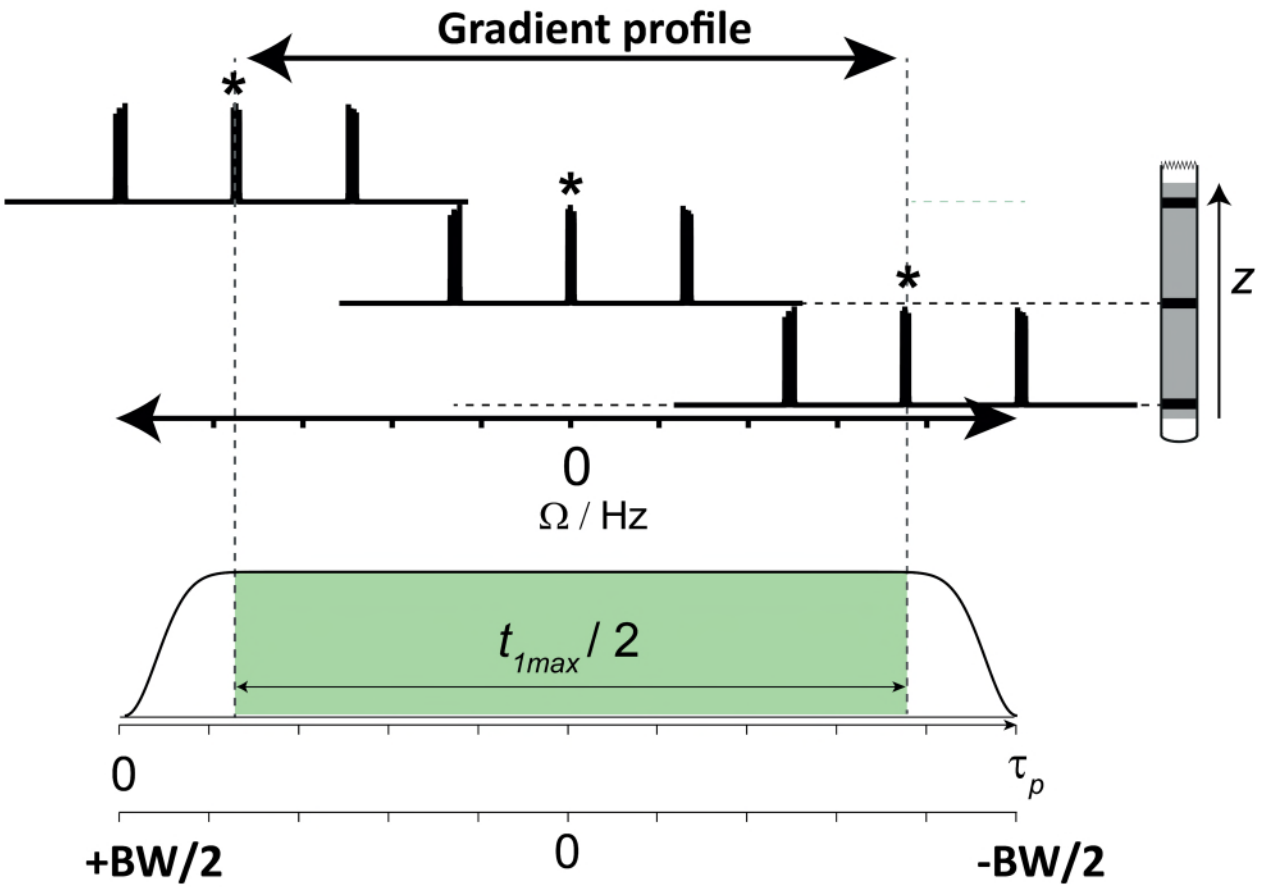
AMX Spin System



Effect of a Gradient – AMX Spin System



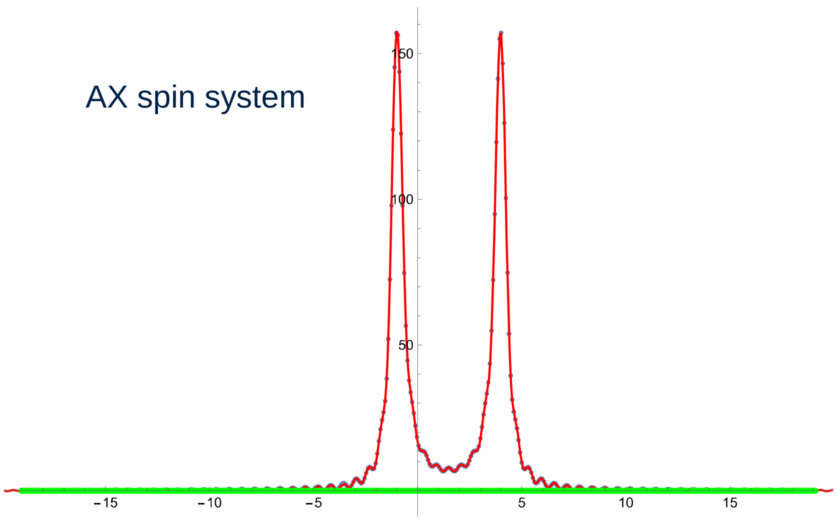
Inversion Error – Second Adiabatic pulse



Error will appear in the top and bottom of the sample

Line shape analysis of doublet

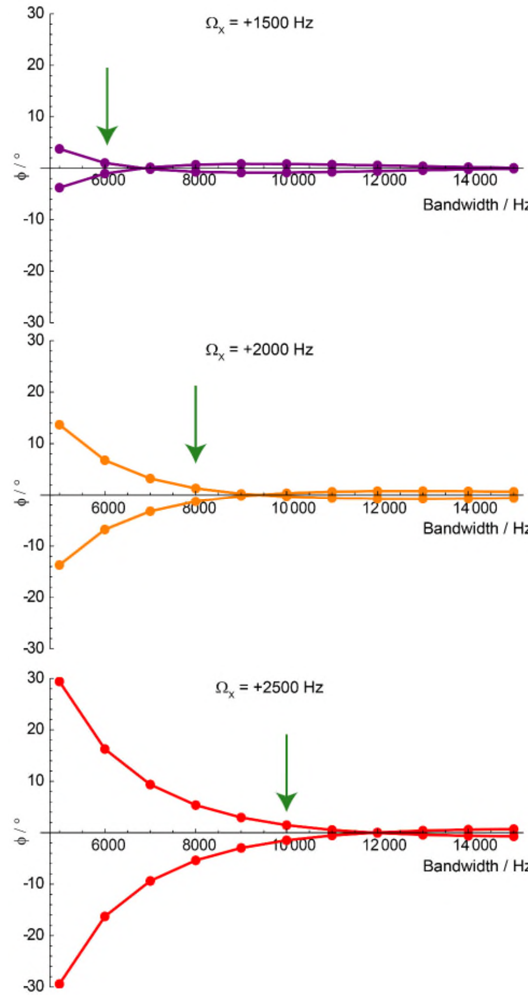
Measure phase of observed multiplet lines as a function of pulse bandwidth



Appropriate SW_G was used for each bandwidth

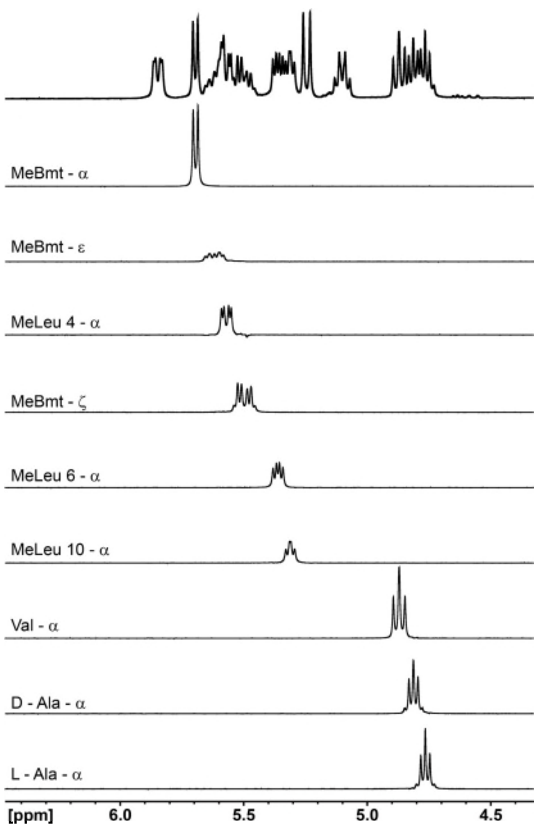
Hz

Phases of doublet components extracted



Cyclosporin A Hα Region

Rule of thumb: use pulse with bandwidth 3 times the frequency range in a given spectrum

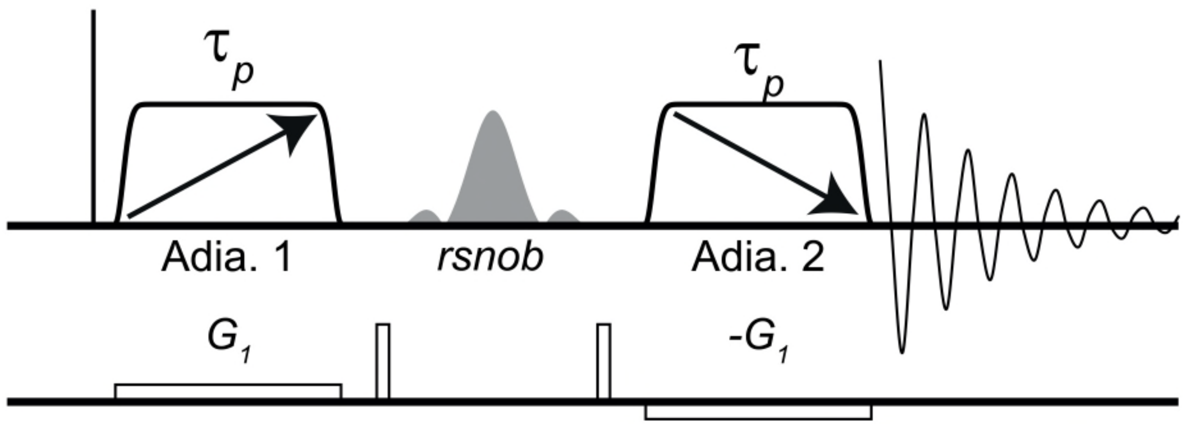


Multiplets are all in phase

Residue	Passive spin Ωs / Hz
Me-Bmt 1 Hα	Hβ -600
Me-Bmt 1 Hε	Hδ -1550 Hζ -50
Me-Leu 4 Hα	2x Hβ -1600
Me-Bmt 1 Hζ	Hε +50 Hη -1500
Me-Leu 6 Hα	Hβ -1250
Me-Leu 10 Hα	Hβ -1250
Val 5 Hα	NH +1050 Hβ -900
D-Ala 7 Hα	NH +1100 Hβ -1500
L-Ala 8 Hα	NH +1300 Hβ -1250

Spectra acquired @ 400 MHz

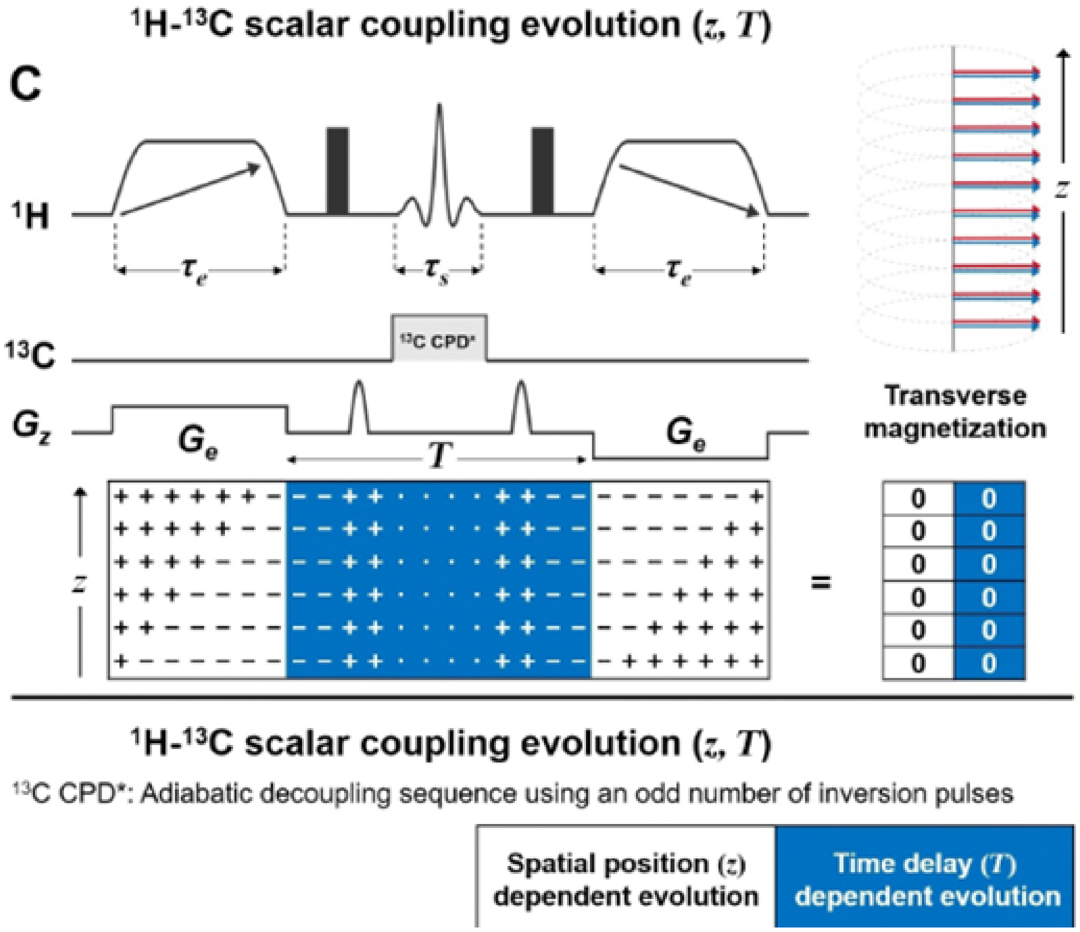
Note on Heteronuclear Couplings



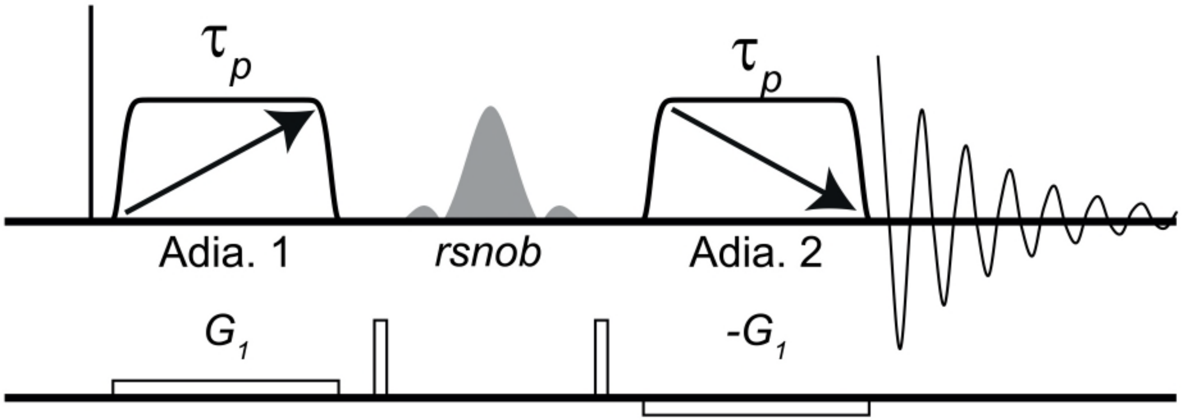
δ	+	-	-	+	+	-
J_{HH}	+	+	+	-	-	-
J_{HX}	+	-	-	+	+	-

Heteronuclear couplings behave like chemical shifts

Heteronuclear coupling solution



A General Parameter Set – ≤ 500 MHz



***Rsnob*:**

- ✓ Must discriminate between active (targeted) and coupled spins
- ✓ Must be wide enough to cover full multiplet width
- ✓ Typically has a 50-100 Hz bandwidth

***Frequency swept pulses*:**

- ✓ High enough bandwidth to minimise imperfect inversion of passive spins
- ✓ Long enough duration so only targeted chemical shift appears in central lobe of sinc function
- ✓ Typically 100 ms duration and 10 kHz bandwidth

Pulse sequences

GEMSTONE Experiments

https://www.nmr.chemistry.manchester.ac.uk/?q=node/255

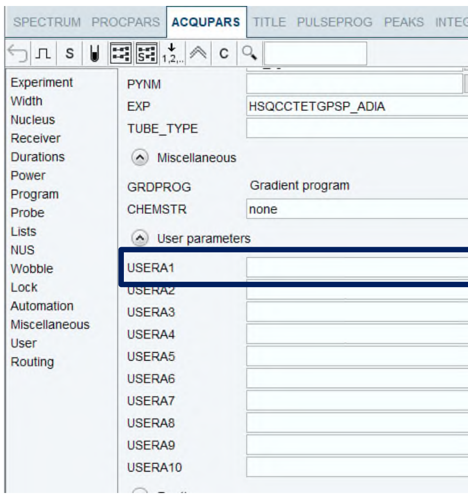
Setting GEMSTONE Pulse Parameters

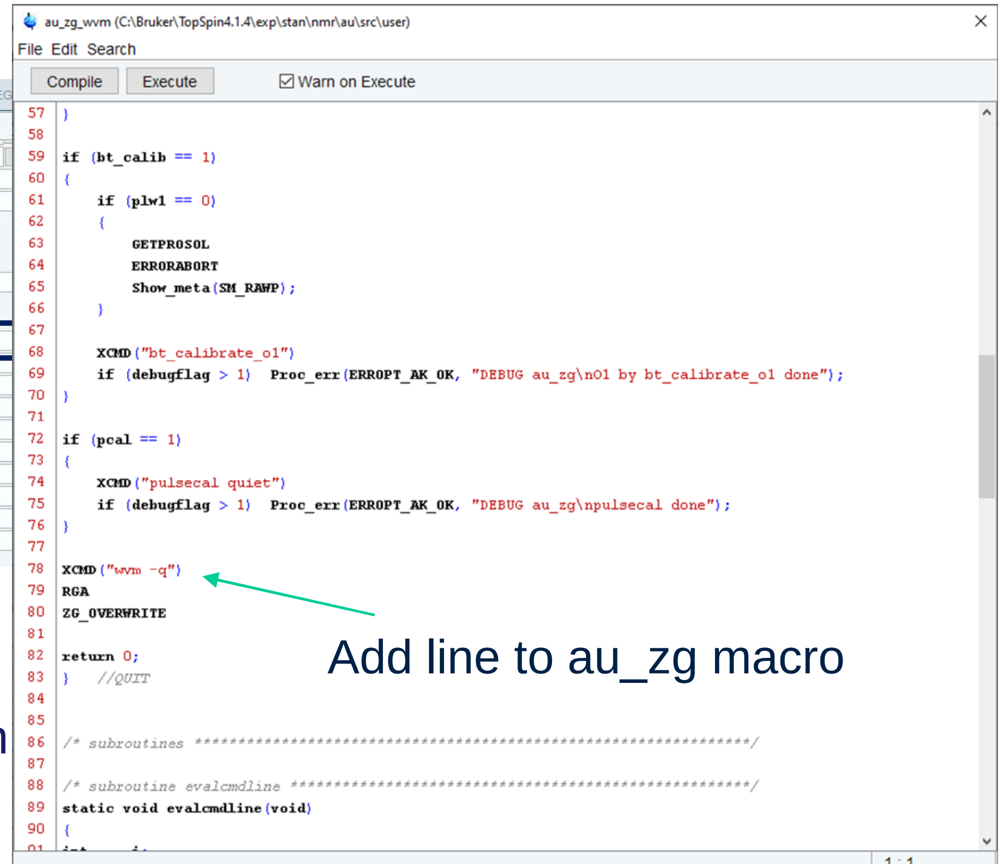
Easy adjustment of GEMSTONE waveforms using wavemaker

General

PULPROG	GEMSTONE		Pulse program for acquisition
TD	65536		Time domain size
SWH [Hz, ppm]	7812.50	19.5248	Sweep width in acquisition direction
AQ [sec]	4.1943040		Acquisition time
RG	101		Receiver gain
DW [μsec]	64.000		Dwell time
DE [μsec]	6.50		Pre-scan-delay
CNST50	100.0000000		Band-width of the band-selective RSNOB pulse [Hz]
CNST51	10000.0000000		Sweep-width of the adiabatic pulse [Hz]
CNST52	100.0000000		Duration of the adiabatic pulse [t1max/2: 30-100 ms]
D1 [sec]	2.000000000		Relaxation delay; 1-5 * T1
d11 [sec]	0.0299999993		d11=30m
D16 [sec]	0.001000000		Delay for homospoil/gradient recovery
DS	2		Number of dummy scans
NS	2		Scans to execute
TD0	1		Number of averages in 1D

Setting GEMSTONE Pulse Parameters



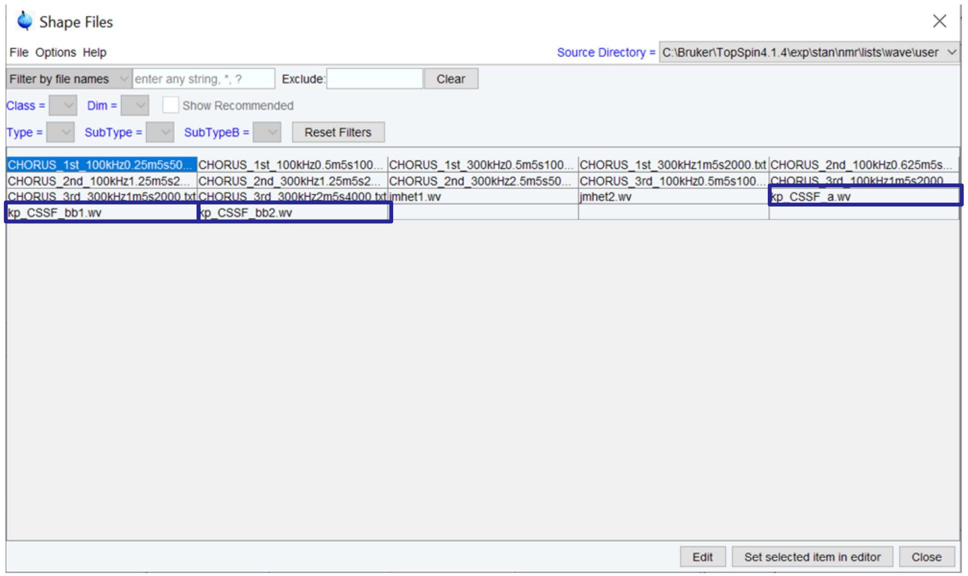


Add line to au_zg macro

Execute “***wvm –q***” in

Prevents pop-up wavemaker output window

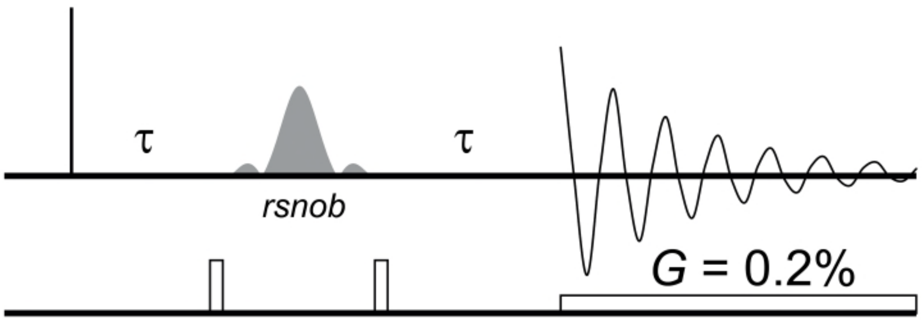
Waveform File Location



“C:\Bruker\TopSpin4.1.4\exp\stan\nmr\lists\wave\user\”

SPNAM 2	kp_CSSF_a.wv	File name for SP2
SPOAL2	0.500	Phase alignment of freq. offset in SP2
SPOFFS2 [Hz]	0	Offset frequency for SP2
SPW2 [W]	0.00038076	F1 channel - shaped pulse
SPNAM 41	kp_CSSF_bb1.wv	File name for SP41
SPOAL41	0.500	Phase alignment of freq. offset in SP41
SPOFFS41 [Hz]	0	Offset frequency for SP41
SPW41 [W]	0.0041617	wvm:kp_CSSF_bb1:f1 wurst-80(cnst51 Hz, cnst52 ms; L2H, Q=11) ss=5.0us;
SPNAM 42	kp_CSSF_bb2.wv	File name for SP42
SPOAL42	0.500	Phase alignment of freq. offset in SP42
SPOFFS42 [Hz]	0	Offset frequency for SP42
SPW42 [W]	0.0041617	wvm:kp_CSSF_bb2:f1 wurst-80(cnst51 Hz, cnst52 ms; H2L, Q=11) ss=5.0us;

GEMSTONE Gradient Strength



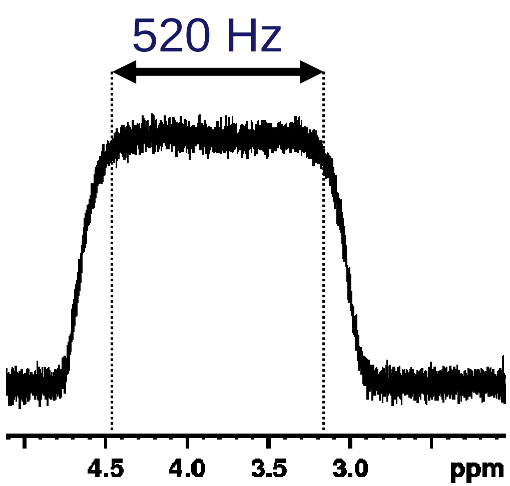
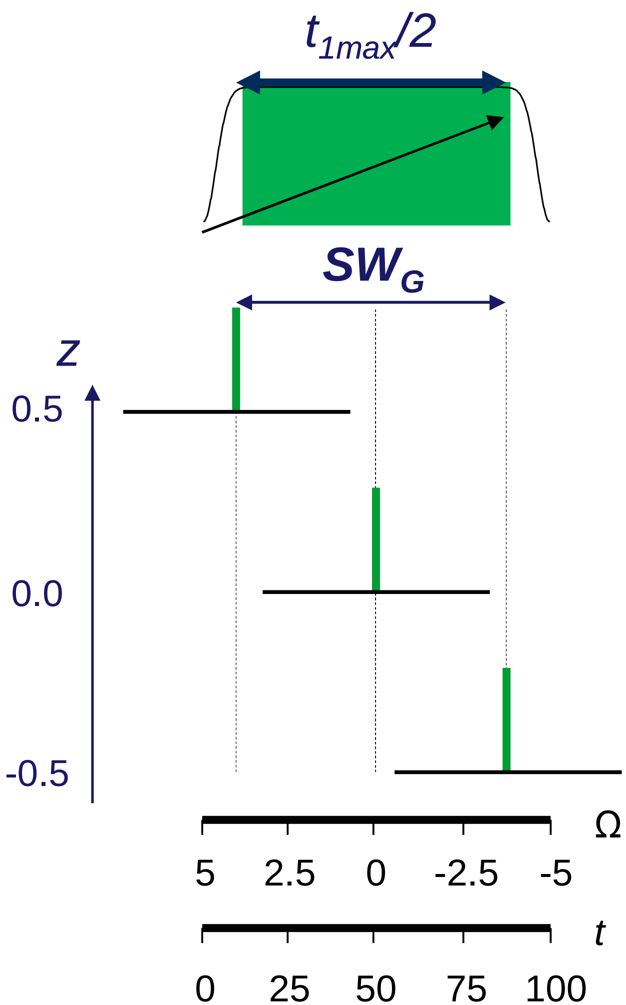


Image of sample



Things to look out for

- Targeted multiplet must be put exactly on resonance
- Strong coupling
- Low viscosity solvents – avoid acetone etc.
- Aliphatic aldehydes
- Other spatio-temporal averaging elements
- Just keep shimming

Acknowledgments

UNIVERSITY OF OXFORD

Peter Kiraly

Ralph Adams

Gareth Morris

Mathias Nilsson

Laura Castañar

Emma Gates

Daniel Taylor

MANCHESTER 1824
The University of Manchester

Over to you

UNIVERSITY OF OXFORD

DISCUSS !!!