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The Basic Building Blocks of NMR Pulse Sequences

James Keeler

University of Cambridge
Department of Chemistry

UNIVERSITY OF CAMBRIDGE

Introduction and outline

- most pulse sequences are built up from simpler elements or building blocks – of which there are not that many
- recognising these elements can help in understanding how a pulse sequence 'works', and will usually simplify a detailed analysis
- new pulse sequences are often designed by joining together these building blocks
- a PDF of this presentation is available to download at `www-keeler.ch.cam.ac.uk`

Introduction and outline

- we will restrict ourselves to building blocks used in liquid-state NMR for scalar coupled spins systems
- everything will be illustrated for two coupled spins one-half
- we will start with a brief reminder of the product operator method, as this will be used to describe each building block
- and then go on to cover as many buildings blocks as time permits
- for more detail: James Keeler, *Understanding NMR Spectroscopy*, 2nd edit., Wiley 2010

Building blocks to be covered

1. Product operators
2. Decoupling
3. Spin echoes
4. Heteronuclear coherence transfer using INEPT
5. Heteronuclear coherence transfer using HMQC
6. Generation and detection of multiple-quantum coherence
7. Constant time sequences
8. Isotropic mixing (TOCSY)
9. z-filters
10. Gradient echoes
11. Spin locking

Product operators

Product operators: one spin

- the state of the spin system can be expressed in terms the nuclear-spin angular momentum operators $\hat{I}_x$, $\hat{I}_y$, and $\hat{I}_z$
- $\hat{I}_x$, $\hat{I}_y$, and $\hat{I}_z$ represent the *x*-, *y*- and *z*-components of the magnetization
- can just 'read off' the expected magnetization
- equilibrium magnetization only along *z*: $\hat{I}_z$

Evolution

- evolution depends on the relevant Hamiltonian
- free precession:

$$\hat{H}_{\text{free}} = \Omega \hat{I}_z$$

  Ω is the offset in the rotating frame

- hard pulse about *x*-axis:

$$\hat{H}_{x,\text{hard pulse}} = \omega_1 \hat{I}_x$$

  ω_1 is the RF field strength

- hard pulse about *y*-axis:

$$\hat{H}_{y,\text{hard pulse}} = \omega_1 \hat{I}_y$$

Arrow notation

- write 'Hamiltonian $\times$ time' over the arrow

$$\text{initial state} \xrightarrow{\text{Hamiltonian} \times \text{time}} \text{final state}$$

- for example, pulse of duration t_{p} about *x* to equilibrium (*z*-) magnetization

$$\hat{I}_z \xrightarrow{\omega_1 t_{\text{p}} \hat{I}_x} \text{final state}$$

- but $\omega_1 t_{\text{p}}$ is the flip angle β

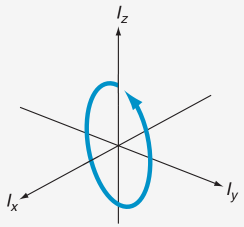
$$\hat{I}_z \xrightarrow{\beta \hat{I}_x} \text{final state}$$

- for example, free evolution of *x*-magnetization for time t

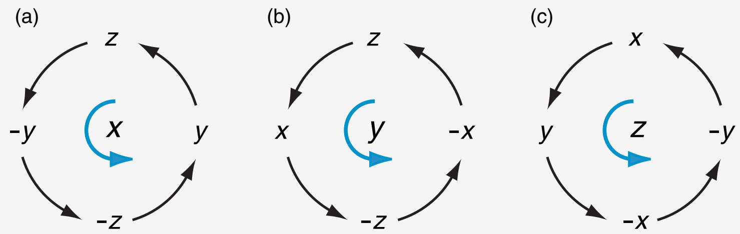
$$\hat{I}_x \xrightarrow{\Omega t \hat{I}_z} \text{final state}$$

Diagrammatic representation of rotations

- in general the rotation of an operator $\hat{A}$ gives two terms:
  1. $\hat{A}$ multiplied by the cosine of an angle
  2. a 'new' operator, $\hat{B}$, multiplied by the sine of the same angle
- $\cos\theta \times$ original operator $+ \sin\theta \times$ new operator
- can work out what the 'new operator' is by looking at the diagram



Diagrammatic representation of rotations



rotation about *x*   rotation about y   rotation about *z*

can be used to determine the effect of any rotation

$\cos\theta \times$ original operator $+ \sin\theta \times$ new operator

90° or 180° rotations particularly simple: just move one or two steps around the clock

Operators for two spins

description	operator(s)
z-magnetization on spin 1	$\hat{I}_{1z}$
in-phase *x*- and *y*-magnetization on spin 1	$\hat{I}_{1x}, \hat{I}_{1y}$
z-magnetization on spin 2	$\hat{I}_{2z}$
in-phase *x*- and *y*-magnetization on spin 2	$\hat{I}_{2x}, \hat{I}_{2y}$
anti-phase *x*- and *y*-magnetization on spin 1	$2\hat{I}_{1x}\hat{I}_{2z}, 2\hat{I}_{1y}\hat{I}_{2z}$
anti-phase *x*- and *y*-magnetization on spin 2	$2\hat{I}_{1z}\hat{I}_{2x}, 2\hat{I}_{1z}\hat{I}_{2y}$
multiple-quantum coherence	$2\hat{I}_{1x}\hat{I}_{2x}, 2\hat{I}_{1x}\hat{I}_{2y}, 2\hat{I}_{1y}\hat{I}_{2x}, 2\hat{I}_{1y}\hat{I}_{2y}$
non-equilibrium population	$2\hat{I}_{1z}\hat{I}_{2z}$

Generation of anti-phase terms by evolution of coupling

- Hamiltonian for coupling: $2\pi J_{12}\hat{I}_{1z}\hat{I}_{2z}$
- evolution of $\hat{I}_{1x}$ under coupling from time τ

$$\hat{I}_{1x} \xrightarrow{2\pi J_{12}\tau\hat{I}_{1z}\hat{I}_{2z}} \cos(\pi J_{12}\tau)\,\hat{I}_{1x} + \sin(\pi J_{12}\tau)\,2\hat{I}_{1y}\hat{I}_{2z}$$

- evolution of *in-phase* (along *x*) to *anti-phase* (along *y*) magnetization
- complete conversion to anti-phase when $\tau = 1/(2J_{12})$

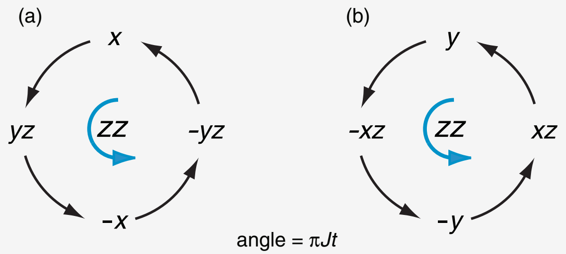
Anti-phase terms evolve back into in-phase terms

- evolution of $2\hat{I}_{1y}\hat{I}_{2z}$ under coupling from time τ

$$2\hat{I}_{1y}\hat{I}_{2z} \xrightarrow{2\pi J_{12}\tau\hat{I}_{1z}\hat{I}_{2z}} \cos(\pi J_{12}\tau)\, 2\hat{I}_{1y}\hat{I}_{2z} - \sin(\pi J_{12}\tau)\,\hat{I}_{1x}$$

- complete conversion to in-phase when $\tau = 1/(2J_{12})$
- note anti-phase along *y* goes to in-phase along $-x$

Diagrammatic representation: evolution of coupling



$$2\hat{I}_{1y}\hat{I}_{2z} \xrightarrow{2\pi J_{12}t\hat{I}_{1z}\hat{I}_{2z}} \cos(\pi J_{12}t)\, 2\hat{I}_{1y}\hat{I}_{2z} - \sin(\pi J_{12}t)\,\hat{I}_{1x}$$

note that the angle is $\pi J t$

Coherence transfer

- an absolutely key concept in multiple-pulse NMR
- achieved by applying 90° pulse to anti-phase term

$$\underbrace{2\hat{I}_{1y}\hat{I}_{2z}}_{\text{on spin 1}} \xrightarrow{(\pi/2)\hat{I}_{1x}} 2\hat{I}_{1z}\hat{I}_{2z} \xrightarrow{(\pi/2)\hat{I}_{2x}} \underbrace{-2\hat{I}_{1z}\hat{I}_{2y}}_{\text{on spin 2}}$$

- *only* anti-phase terms are transferred
- anti-phase terms arise due to the evolution of coupling

Decoupling

Heteronuclear broadband decoupling

- a broad-band decoupling sequence applied to the *I* spins effectively sets all heteronuclear couplings to the *S*-spins to zero
- the following are likely to be dephased:
  1. *I*-spin coherences (including heteronuclear multiple quantum)
  2. anti-phase terms on *I* (e.g. $2\hat{I}_z\hat{S}_x$)
  3. *z*-magnetization on *I*

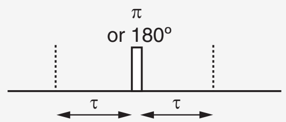
  – unless decoupling only applied for a short period
- broad-band sequences gives decoupling over wide range of *I*-spin shifts with minimum of power, but there are practical limits to the range that can be covered

Semi-selective decoupling

- by deliberately reducing the RF power level it is possible to restrict the decoupling effect to a narrower range of shifts
- e.g. decoupling of only the carbonyl carbons, or the α-carbons
- only likely to be successful for a group of resonances which is well-separated from others

Spin echoes

Spin echo for one spin



- start with $\hat{I}_x$

$$\hat{I}_x \xrightarrow{\Omega\tau\hat{I}_z} \cos(\Omega\tau)\hat{I}_x + \sin(\Omega\tau)\hat{I}_y$$

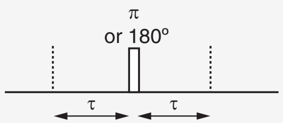
- 180° pulse about *x* does not affect $\hat{I}_x$, and inverts $\hat{I}_y$

$$\cos(\Omega\tau)\hat{I}_x + \sin(\Omega\tau)\hat{I}_y \xrightarrow{\pi\hat{I}_x} \cos(\Omega\tau)\hat{I}_x - \sin(\Omega\tau)\hat{I}_y$$

- second delay

$$\begin{aligned}\cos(\Omega\tau)\hat{I}_x - \sin(\Omega\tau)\hat{I}_y \xrightarrow{\Omega\tau\hat{I}_z} & \cos(\Omega\tau)\cos(\Omega\tau)\hat{I}_x + \sin(\Omega\tau)\cos(\Omega\tau)\hat{I}_y \\ & - \cos(\Omega\tau)\sin(\Omega\tau)\hat{I}_y + \sin(\Omega\tau)\sin(\Omega\tau)\hat{I}_x \\ & \equiv \hat{I}_x\end{aligned}$$

Spin echo for one spin



- evolution between the dashed lines is

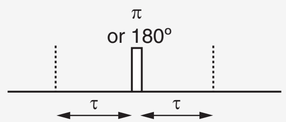
$$\hat{I}_x \xrightarrow{\tau-\pi-\tau} \hat{I}_x$$

- the offset is said to be *refocused* between the dashed lines
i.e. it is as if the offset (or the delay) is zero

- works just as well with other initial states

- in fact $\tau - \pi - \tau \equiv \pi$

Spin echo for (homonuclear) coupled spins



- start with $\hat{I}_{1x}$ and assume that offset is refocused

- the 180° pulse affects both spins . . .

- . . . final result is

$$\cos(2\pi J_{12}\tau)\hat{I}_{1x} + \sin(2\pi J_{12}\tau)2\hat{I}_{1y}\hat{I}_{2z}$$

- coupling is *not refocused*, but continues to evolve for the *entire period* 2τ

Spin echo for (homonuclear) coupled spins

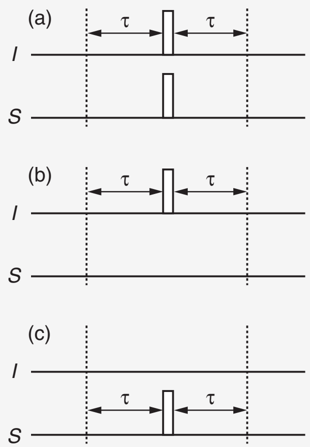
- a spin echo is *equivalent* to
  1. evolution of the coupling for time 2τ
  2. followed by a 180° pulse (here about *x*).

- this is a very useful short cut in calculations

- key thing about a spin echo is that it enables us to interconvert in-phase and anti-phase magnetization in a way which is *independent* of the offset i.e. *works for all spins*

Spin echoes in heteronuclear spin systems

can choose which spins to apply 180° pulses to



- (a) offsets refocused, coupling not refocused (as homonuclear)

- (b) only *I*-spin offset refocused, coupling refocused

- (c) only *S*-spin offset refocused, coupling refocused

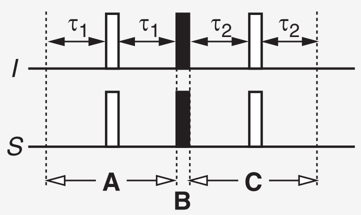
gives considerable flexibility

Spin echoes: summary

- in **homonuclear** systems spin echoes allow the coupling to evolve while effectively suppressing the evolution due to the offset (shift)
- means that we can interconvert in-phase and anti-phase terms in a way which is *independent of the offset*
- in **heteronuclear** systems spin echoes can effectively suppress the evolution of heteronuclear couplings, which is equivalent to ‘decoupling’, independent of offset

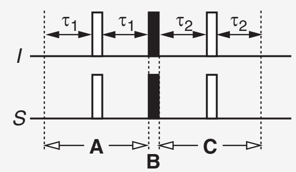
Heteronuclear coherence transfer using INEPT

INEPT transfer



- start with in-phase magnetization on *I* e.g. $\hat{I}_x$
- anti-phase generated during spin echo **A** (independent of offset)
- coherence transfer from *I* to *S* during pulses **B**
- transferred anti-phase goes in-phase during spin echo **C** (independent of offset)
- overall result is transfer of in-phase on *I* to in-phase on *S*

INEPT transfer



- starting with $\hat{I}_x$, first echo gives

$$\xrightarrow{\tau_1-\pi-\tau_1} \cos(2\pi J_{IS}\tau_1)\,\hat{I}_x + \sin(2\pi J_{IS}\tau_1)\,2\hat{I}_y\hat{S}_z$$

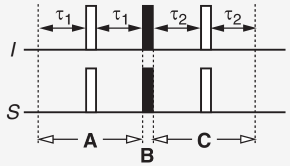
- only the anti-phase term is transferred by the pulses

$$\sin(2\pi J_{IS}\tau_1)\,2\hat{I}_y\hat{S}_z \xrightarrow{(\pi/2)\hat{I}_x} \xrightarrow{(\pi/2)\hat{S}_x} -\sin(2\pi J_{IS}\tau_1)\,2\hat{I}_z\hat{S}_y$$

- anti-phase term goes in-phase during second spin echo

$$\xrightarrow{\tau_2-\pi-\tau_2} \sin(2\pi J_{IS}\tau_2)\sin(2\pi J_{IS}\tau_1)\,\hat{S}_x$$

INEPT transfer



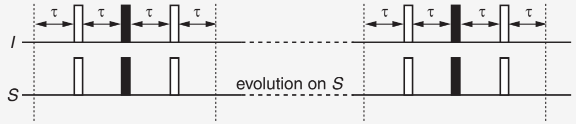
- overall result is

$$\hat{I}_x \xrightarrow{\text{INEPT}} \sin(2\pi J_{IS}\tau_2)\sin(2\pi J_{IS}\tau_1)\,\hat{S}_x$$

- maximum transfer when $\tau_1 = 1/(4J_{IS})$ and $\tau_2 = 1/(4J_{IS})$
- if anti-phase term present at start of **A**, can omit first spin echo
- phase of 90° pulse to *I* affects which term is transferred i.e. $2\hat{I}_y\hat{S}_z$ or $2\hat{I}_x\hat{S}_z$

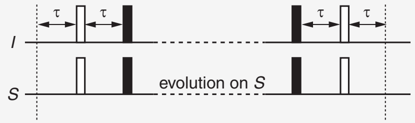
Double INEPT transfer

- transfer from *I* to *S*; evolution; transfer back from *S* to *I*



$$\hat{I}_x \xrightarrow{\text{double INEPT}} \text{mod. from period on } S \text{ spin} \times \sin(2\pi J_{IS}\tau)^4\,\hat{I}_x$$

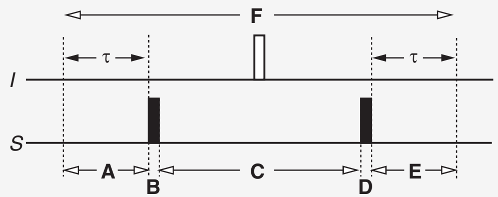
- can omit middle two spin echoes if evolution of an anti-phase term is acceptable



$$\hat{I}_x \xrightarrow{\text{double INEPT}} \text{mod. from period on } S \text{ spin} \times \sin(2\pi J_{IS}\tau)^2\,\hat{I}_x$$

Heteronuclear coherence transfer using HMQC

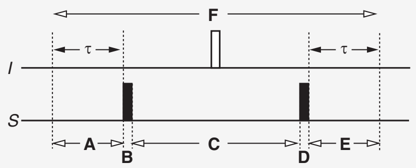
Coherence transfer using heteronuclear multiple quantum



- rather like double INEPT
- *I*-spin offset refocused over whole of period **F** (spin echo due to central 180° pulse)
- start with $\hat{I}_x$: goes anti-phase during τ to give $\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_z$
- *S*-spin 90° pulse transfers this to heteronuclear multiple-quantum coherence (HMQC)

$$\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_z \xrightarrow{(\pi/2)\hat{S}_x} -\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_y$$

Coherence transfer using HMQC



- HMQC does not evolve under J_{IS}; I-spin offset also refocused
- evolution under just S-spin offset for period **C**

$$-\cos(\Omega_S t)\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_y + \sin(\Omega_S t)\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_x$$

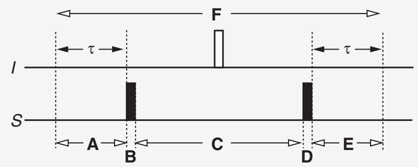
- 90° pulse to S transfers only the first term to give

$$-\cos(\Omega_S t)\sin(\pi J_{IS}\tau)\,2\hat{I}_y\hat{S}_z$$

- this goes in-phase during the final delay τ to give

$$\cos(\Omega_S t)\sin(\pi J_{IS}\tau)\sin(\pi J_{IS}\tau)\,\hat{I}_x$$

Coherence transfer using HMQC



- net result is

$$\hat{I}_x \xrightarrow{\text{HMQC transfer}} \text{mod. from } S\text{-spin} \times \sin^2(\pi J_{IS}\tau)\,\hat{I}_x$$

- optimum delay τ is $1/(2J_{IS})$
- similar to double INEPT

Generation and detection of multiple-quantum coherence

Generation of multiple quantum coherence (MQC)

- simply:

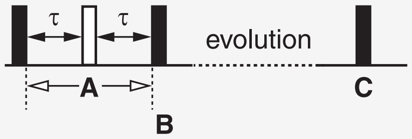
$$\underbrace{2\hat{I}_{1x}\hat{I}_{2z}}_{\text{anti-phase on spin 1}} \xrightarrow{(\pi/2)(\hat{I}_{1x}+\hat{I}_{2x})} \underbrace{-2\hat{I}_{1x}\hat{I}_{2y}}_{\text{MQC}}$$

- *only* anti-phase terms are converted to MQC
- MQC not directly observable, but can be transferred back to anti-phase with a further pulse

$$2\hat{I}_{1x}\hat{I}_{2y} \xrightarrow{\text{evolution of offset}} \text{mixture of terms: } 2\hat{I}_{1x}\hat{I}_{2y},\ 2\hat{I}_{1y}\hat{I}_{2y},\ 2\hat{I}_{1x}\hat{I}_{2x},\ 2\hat{I}_{1y}\hat{I}_{2x}$$

- only $2\hat{I}_{1x}\hat{I}_{2y}$ and $2\hat{I}_{1y}\hat{I}_{2x}$ transferred back to observable anti-phase by 90°(x) pulse

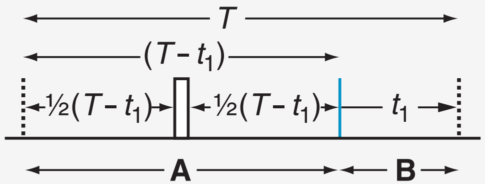
Typical MQC sequence



- anti-phase generated during spin echo **A**
- MQC generated by pulse **B**
- evolution
- anti-phase regenerated by pulse **C**; could add a further spin echo to re-phase

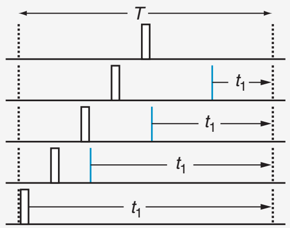
Constant time sequences

Constant time (CT) pulse sequence element



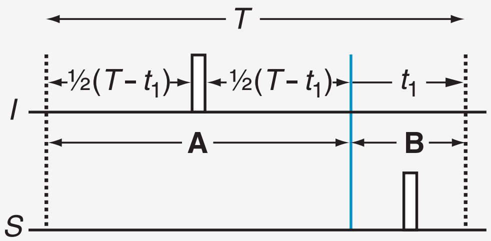
- a way of suppressing the splittings in an *indirect* dimension due to *homonuclear* coupling
- the time T is **fixed**, but the position of the 180° pulse changes with t_1
- coupling evolves for the entire time T which, as it is constant, means that the signal is *not modulated by the coupling*
- offset refocused over period **A**, but evolves over period **B** ($\equiv t_1$), so signal is modulated by offset

CT pulse sequence element - in action



- 180° pulse moves backwards as t_1 increases
- problems:
  1. relative amounts of in-phase and anti-phase at end of sequence depend on the size of the couplings and the time T
  2. it may be difficult to find a value of T which gives reasonable amounts of the required terms
  3. the maximum value of t_1 is limited by T
  4. signal relaxes for entire time T, even when $t_1 = 0$: loss of intensity

CT pulse sequence element: heteronuclear case



- *I*-spin 180° pulse refocuses heteronuclear coupling over period **A**
- *S*-spin 180° pulse refocuses heteronuclear coupling over period **B** (t_1)
- can re-introduce modulation due to heteronuclear coupling by displacing the *S*-spin pulse from the centre of period **B**

Isotropic mixing (TOCSY)

Isotropic mixing

- a specially designed pulse sequence (e.g. DIPSI) can, over a range of offsets, generate the effective Hamiltonian

$$\hat{H}_{\text{iso}} = \sum_{\text{all pairs } i,j} 2\pi J_{ij}\, \hat{\mathbf{I}}_i \,.\, \hat{\mathbf{I}}_j$$

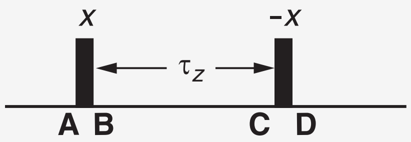
- this is the full version of the Hamiltonian for coupling (not truncated for weak coupling)
- $\hat{H}_{\text{iso}}$ is invariant to rotations – hence described as *isotropic*
- DIPSI involves a continuous train of pulses with variable flip angles and phases; issue over power deposition

Isotropic mixing

- the effect of $\hat{H}_{\text{iso}}$ is to transfer magnetization (along *x*, *y* or *z*) from one spin to any other spin *connected by an unbroken chain of couplings*
- rate of transfer depends on the size of the coupling constants; for a two-spin system transfer goes as $\sin^2(\pi J_{12}\tau)$
- transfer also results in generation of anti-phase terms from $\hat{I}_{1x}$ or $\hat{I}_{1y}$, and zero-quantum coherence from $\hat{I}_{1z}$
- need to ‘clean up’ the transfer e.g. using a *z*-filter

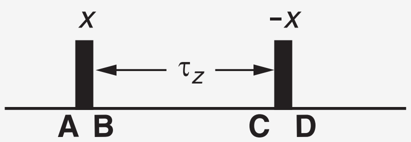
z-filters

Aim of z filter



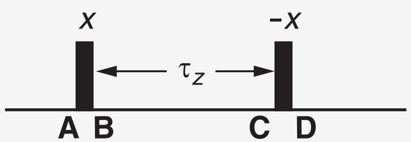
- suppose that at point **A** we have a mixture of terms, but wish to select just one in-phase term such as $\hat{I}_{1y}$
- $90°(x)$ rotates $\hat{I}_{1y}$ to $\hat{I}_{1z}$; other terms rotated in various ways
- use phase cycling or gradient pulses to select **just** z-magnetization at point **C**
- $90°(-x)$ pulse rotates $\hat{I}_{1z}$ to $\hat{I}_{1y}$ at point **D**
- effectively selects $\hat{I}_{1y}$ at **A** by passing through z-magnetization

Problem with z filter



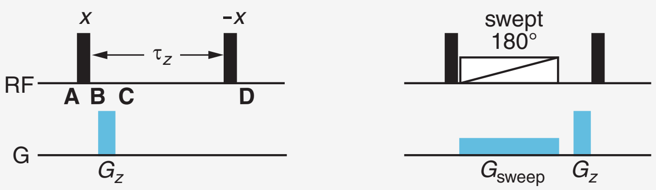
- some anti-phase terms at **A** are transferred to zero-quantum coherence (ZQC) at point **B**
- ZQC is not distinguishable from z-magnetization by phase cycling or gradient pulses: it is not suppressed, therefore
- ZQC at **C** becomes anti-phase magnetization at **D**, making z-filter ineffective

Problem with z filter: possible solution



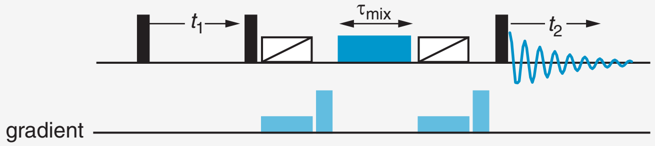
- ZQC evolves for time τ_z, thus acquiring a phase
- co-addition of the results of experiments with *different* τ_z values will result in partial cancellation of contributions arising from ZQC
- τ_z needs to range up to a value comparable with 1/(2 $\times$ zero-quantum frequency); difficult to arrange for a complex spin system
- repetition with different τ_z values is time consuming

z filter with swept gradient



- use gradient G_z to dephase all but z-magnetization and ZQC
- use weak gradient in combination with swept-frequency 180° pulse to suppress ZQC (see Chapter 11, Section 11.15.2)
- one-shot z-filter: no phase cycling or repetition needed

TOCSY with double z filter



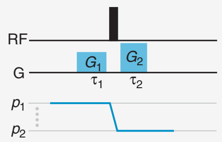
- isotropic mixing embedded in z-filter
- selection of z-magnetization before and after mixing
- ensures that only y-magnetization present before second 90° pulse and after third 90° pulse contributes to spectrum
- gives in-phase multiplets with absorption-mode lineshapes

Gradient echoes

Field gradient pulses

- normally we try to make the B_0 field as homogeneous as possible . . . but it can be advantageous to introduce controlled inhomogeneity
- a small coil, in the probe, generates a small field which varies linearly along z: a *field gradient*
- can control the *size* of the gradient by varying the current, and the *sign* by altering the direction in which the current flows
- can also control the *duration* of the gradient

Defocusing and refocusing

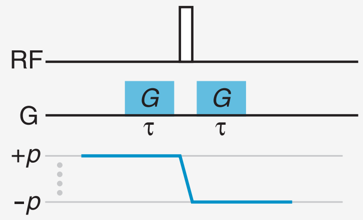


- during first gradient pulse coherence (e.g. transverse magnetization) is dephased
- extent of dephasing depends on the coherence order p, the duration of the gradient τ and its strength G
- after RF pulse, the coherence can be re-phased by applying a second gradient; refocusing condition:

$$\frac{G_1\tau_1}{G_2\tau_2} = -\frac{p_2}{p_1}$$

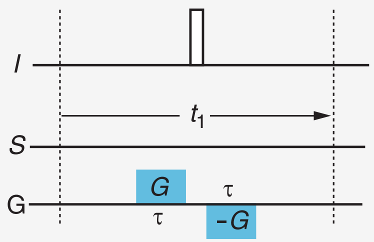
- $p_1 \rightarrow p_2$ pathway refocused; (hopefully) others remain dephased and so are not observed

Refocusing pulses



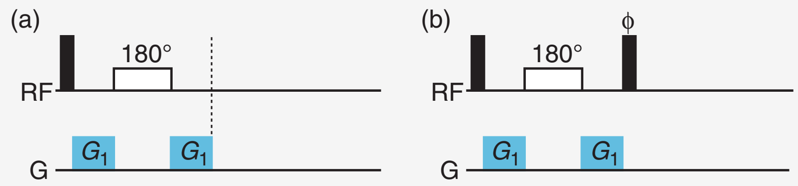
- an ideal 180° pulse causes $p \rightarrow -p$
- selected, *for all p*, by a pair of equal gradients
- if pulse is imperfect, gradient pair 'cleans up' any unwanted transfers
- this *gradient echo* is simple and convenient

'Cleaning up' 180° pulses in heteronuclear experiments



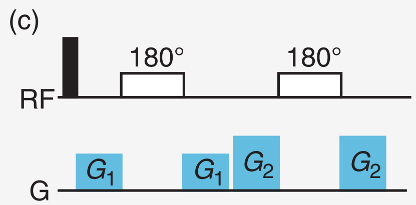
- coherence on *S* but not on *I*: role of 180° pulse is to invert $\hat{I}_z$
- if pulse is imperfect may also generate transverse magnetization: second gradient dephases this
- coherence on *S* dephased by first gradient and rephased by second

Selective excitation with the aid of gradients



- 180° pulse is *selective*
- only the magn. from those spins which experience the selective pulse as a refocusing pulse will be refocused by second gradient
- everything else remains defocused: very clean excitation
- can add extra 90° pulse to flip magnetization onto $-z$: gives *selective inversion*

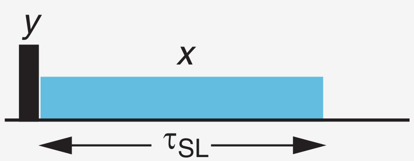
Double pulsed field gradient spin echo



- double pulsed field gradient spin echo (DPFGSE)
- only the magn. from those spins which experience *both* selective pulses as refocusing pulses will be excited
- advantage over single spin echo is that the *phase is constant* over the excited bandwidth
- two gradient pairs must be independent

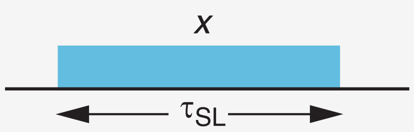
Spin locking

Typical spin locking sequence



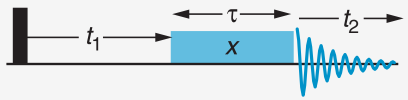
- non-selective pulse puts magnetization along x
- then apply a *strong* RF field along x
- if the RF field strength is greater than typical offsets then evolution due to offsets is suppressed and the magnetization remains 'locked' along x

Features of spin locking



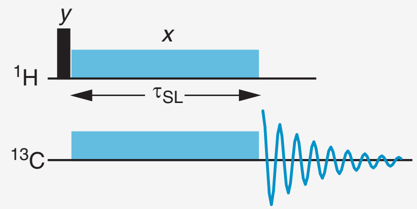
- magnetization not along the spin-lock axis likely to be dephased due to inhomogeneity of RF field
- result is a selection of just the x-magnetization
- if RF field is not large compared to the offset, then the spin-lock axis tilts up in the xz-plane (aligned with the effective field)
- heating of the sample and/or damage to the probe limits the RF power that can be used

Applications: ROESY



- ROESY: detection of cross relaxation between transverse magnetization on different spins i.e. transverse NOE
- useful in cases where conventional NOE is zero
- can also probe relaxation dispersion and chemical exchange by measuring decay of spin-locked magnetization as function of the spin-locking field strength

Applications: cross-polarization in solids



- Hartmann–Hahn cross polarization **in solids** via dipolar coupling
- transfer of magnetization from abundant ^{1}H to low-abundance ^{13}C: need to match field strengths according to $\gamma_I \omega_{1,I} = \gamma_S \omega_{1,S}$
- used for: (a) sensitivity enhancement; (b) transfer of magnetization in multiple-pulse experiments

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The Basic Building Blocks of NMR Pulse Sequences

The End

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