2D NMR part III

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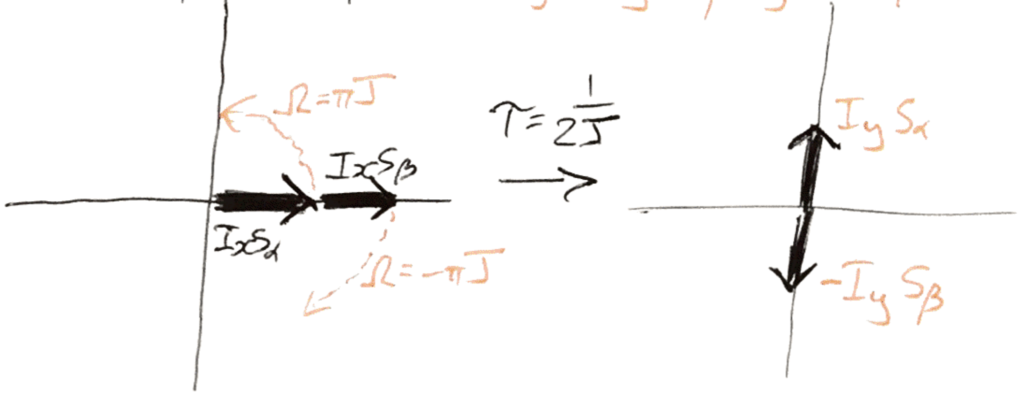
Some common elements of pulse sequences…

- Decoupling
- Constant time evolution
- Selective pulses & Bloch-Siegert shifts
- Adiabatic pulses
- Simple applications of gradient pulses
- Water suppression

Recap: vector model description of J-coupling

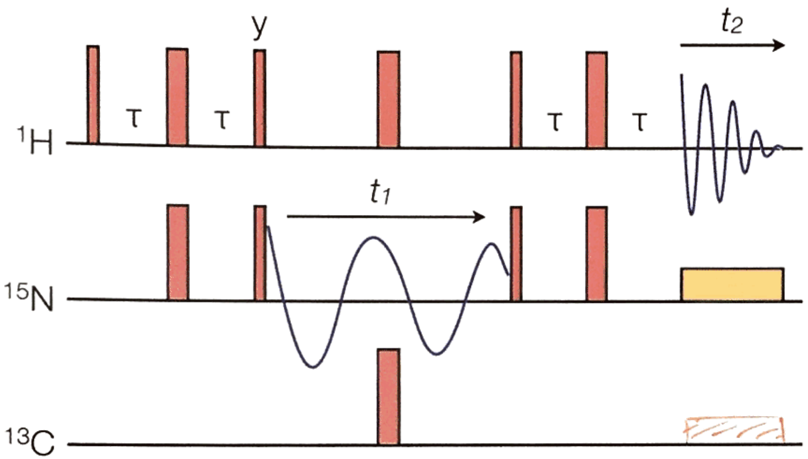
$$\underbrace{I_x = I_x(S_\alpha + S_\beta)}_{I_x \text{ irrespective of } S \text{ spin state}} \longrightarrow \underbrace{I_y(S_\alpha - S_\beta) = I_y S_z}_{\text{Sign change depending on } S \text{ spin state}}$$



Decoupling (on-resonance)

- Coupling = splitting of resonances by frequency J
- Therefore, to observe (resolve) coupling, need to observe for time $\tau \gtrsim 1 / J$
    - i.e. lifetime of coupled state must be $\gtrsim 1 / J$
- Converse: reduce the lifetime, and coupling won't be observed
- Basic idea: exchange S_α <—> S_β with π pulse to refocus coupling evolution

HSQC with ^{13}C decoupling



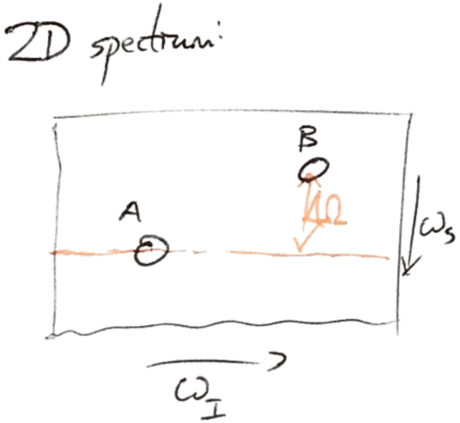
Weak coupling ($^{1}J_{NC'}$ ~ 15 Hz)
Invert ^{13}C spin-state half-way through ^{15}N evolution

Decoupling during acquisition

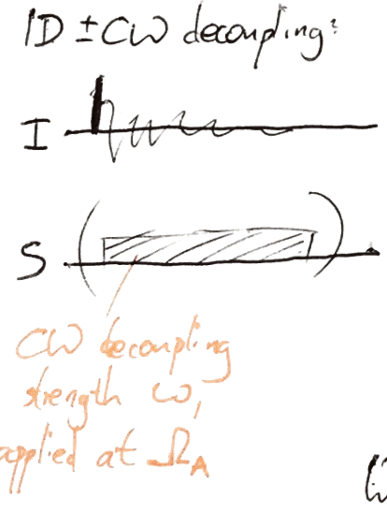
- Unlike decoupling of indirect dimension, magnetisation is observed continuously
  - No longer sufficient to refocus coupling over entire evolution time
  - For effective decoupling, coupling must be continuously refocused on timescale << 1 / J
- J couplings are proportional to the gyromagnetic ratios of the two nuclei – so ^{1}H couplings are much stronger
  - e.g. $^{1}J_{NH} \approx 90$ Hz, τ << 11 ms
  - e.g. $^{1}J_{CH} \approx 140$ Hz, τ << 7 ms
- Heavy rf loads – power limits…
- Quality of inversion? Off-resonance effects…

Off-resonance effects / selective decoupling

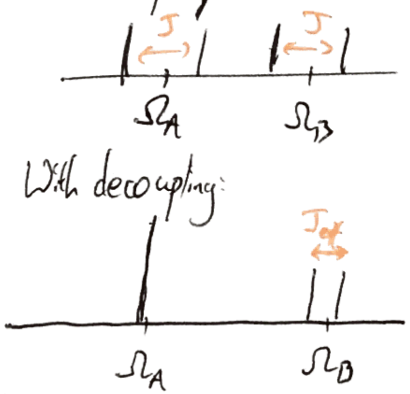
2D spectrum:



1D ± CW decoupling:

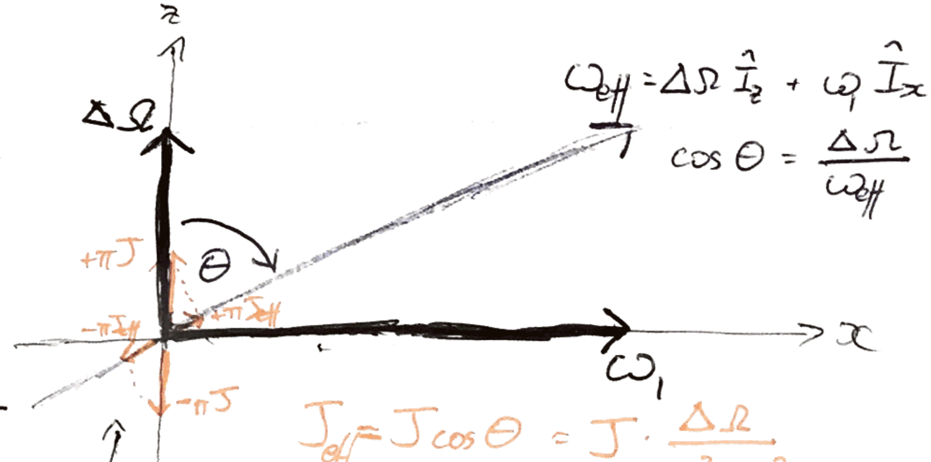


No decoupling:



What's going on with spin B?

Vector model description of J coupling scaling



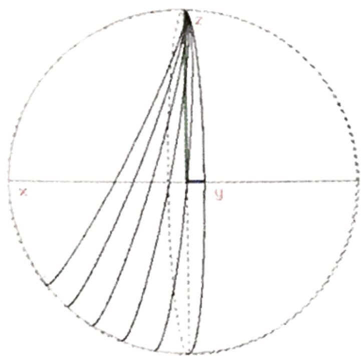
$\omega_{eff} = \Delta\Omega \hat{I}_z + \omega_1 \hat{I}_x$

$\cos\theta = \frac{\Delta\Omega}{\omega_{eff}}$

$J_{eff} = J\cos\theta = J \cdot \frac{\Delta\Omega}{\Delta\Omega^2 + \omega_1^2}$

J coupling is projected onto the effective field

Off-resonance effects of hard pulses



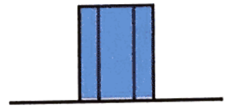
Off-resonance effects
of hard π pulse

Solution 1 – more power?
– hardware limits
– sample heating

Composite pulses

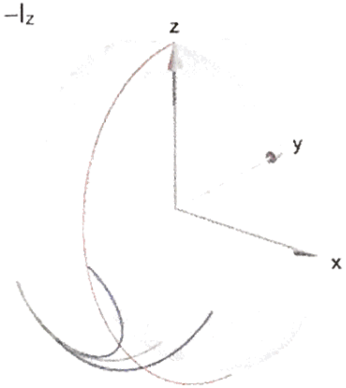
- Correction of:
  - off-resonance effects
  - mis-calibration / B_1 inhomogeneity
- Particularly important for inversion, $I_z \longrightarrow -I_z$

π_x

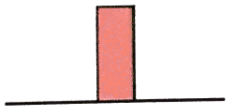


$(\pi/2)_x - \pi_y - (\pi/2)_x$

$(\pi/2)_x - (4\pi/3)_y - (\pi/2)_x$



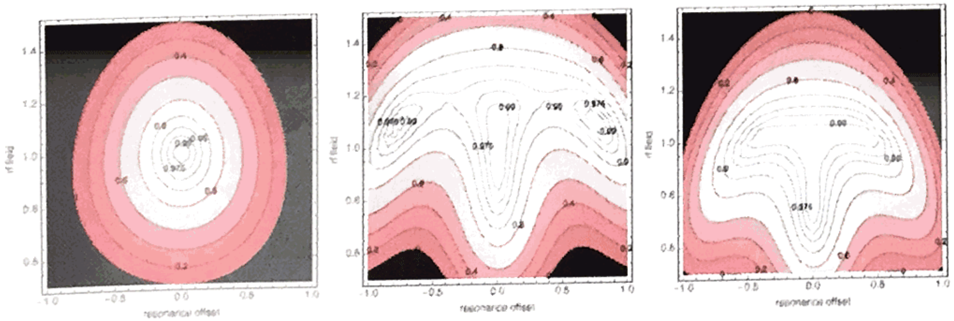
Composite pulse performance



π_x

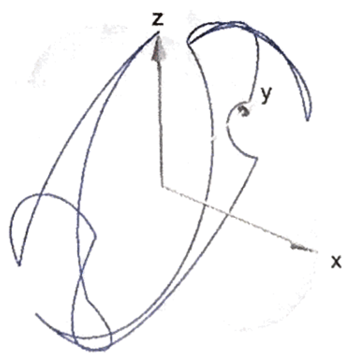
$(\pi/2)_x - \pi_y - (\pi/2)_x$

$(\pi/2)_x - (4\pi/3)_y$
$- (\pi/2)_x$

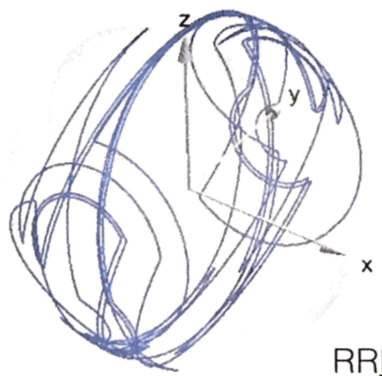


Cycles and supercycles

$R = (\pi/2)_x - \pi_y - (\pi/2)_x$

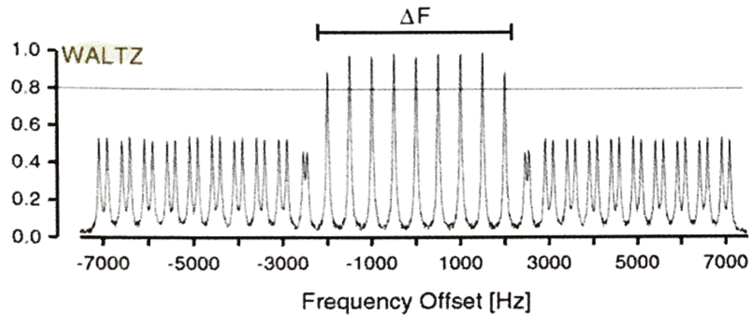


$RR\underline{RR}$



$RR\underline{RR}$
$\underline{R}RR\underline{R}$
$\underline{RR}RR$
$R\underline{RR}R$

WALTZ-16



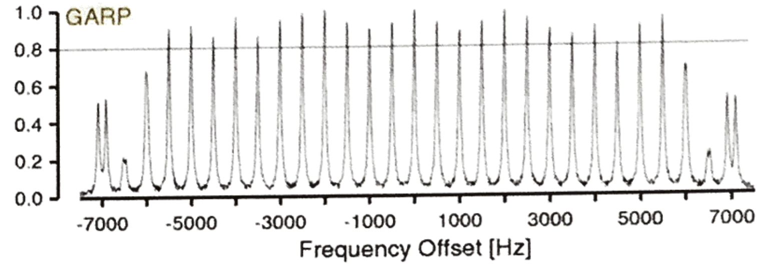
$$\Xi = \frac{2\pi\Delta F}{\gamma B_1} = 1.8$$

Frequency range with at least 80% intensity

'Figure of merit'

Fundamentals of Protein NMR Spectroscopy
By Gordon S. Rule, T. Kevin Hitchens

GARP

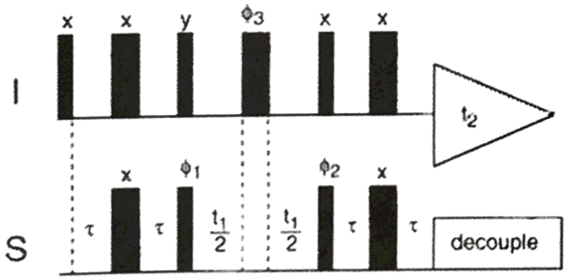


$$\Xi = \frac{2\pi\Delta F}{\gamma B_1} = 4.8$$

- Much bigger bandwidth
- Less uniform intensities

Fundamentals of Protein NMR Spectroscopy
By Gordon S. Rule, T. Kevin Hitchens

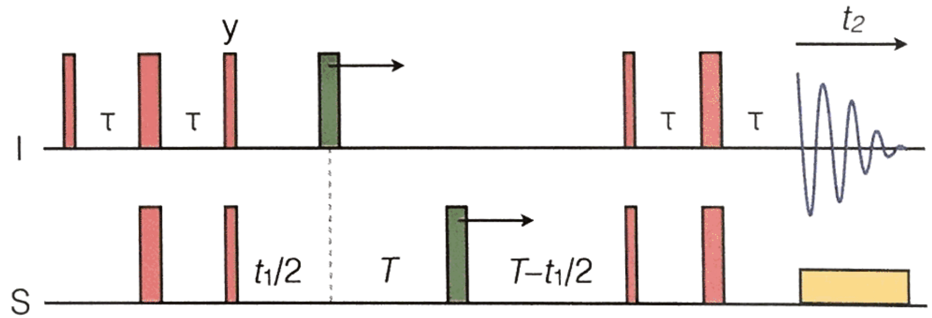
Constant time acquisition



What do you do when there are large homonuclear couplings in the S spins (e.g. $^1J_{CC}$)?

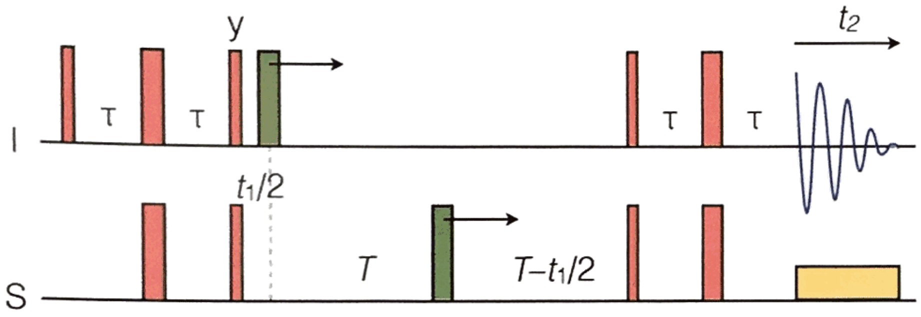
eg. fully labelled ^{13}C sample

Constant time acquisition



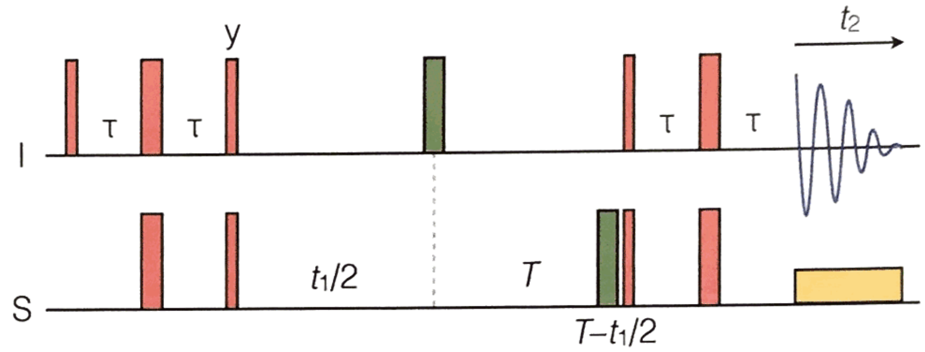
$$2T = n\,{}^1J_{CC}$$

Constant time acquisition



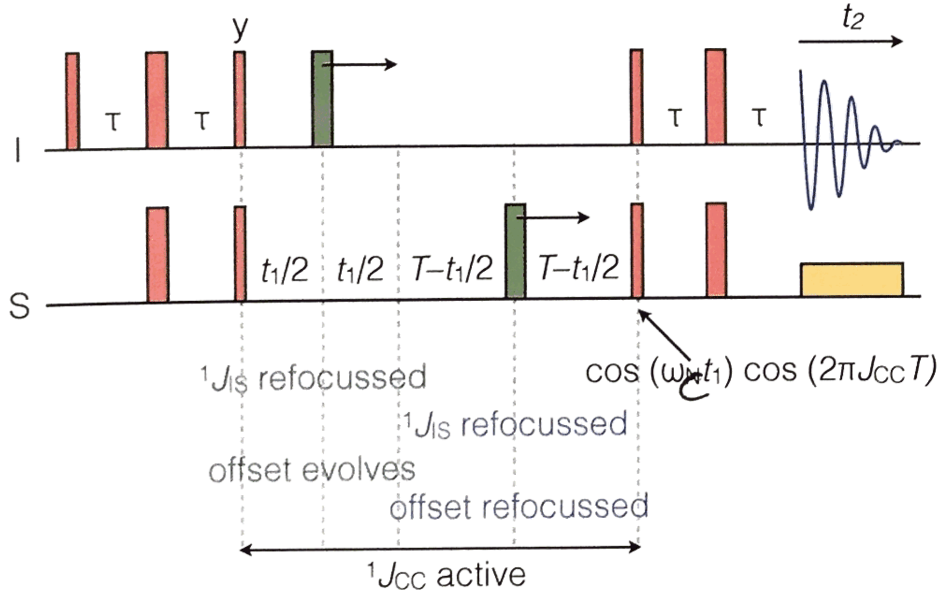
t_1 small: just a spin-echo on ^{15}N
chemical shift refocussed
coupling refocussed (by ^{15}N 180° pulse)

Constant time acquisition



$t_1 = 2T$: maximum possible value of t_1
chemical shift evolves
coupling refocussed (by ^1H 180°)

Constant time acquisition



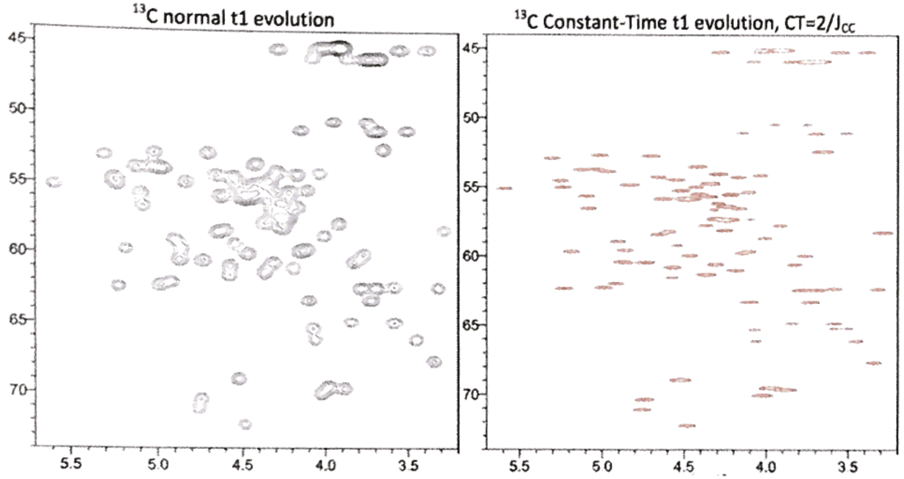
Constant time acquisition

Observed magnetisation proportional to:
$\cos(\omega_{\cancel{N}C} t_1) \cos(2\pi J_{CC} T)$

Choose: $2T = n / {}^1J_{CC}$

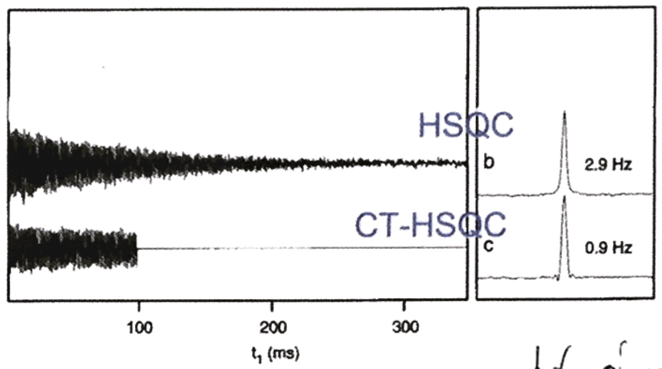
${}^1J_{CC} \sim 35$ Hz, so $2T = 27$ or 54 ms

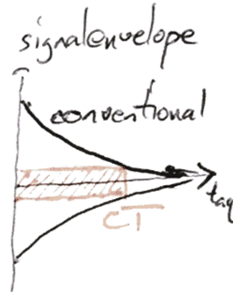
Constant time acquisition



Hα/Cα region of ^{1}H,^{13}C-HSQC of ubiquitin

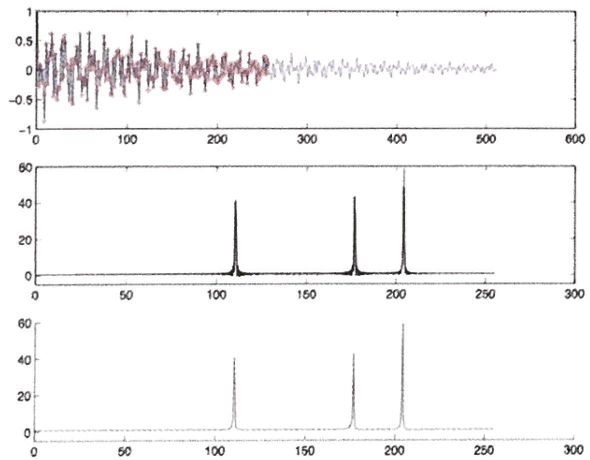
Constant time acquisition





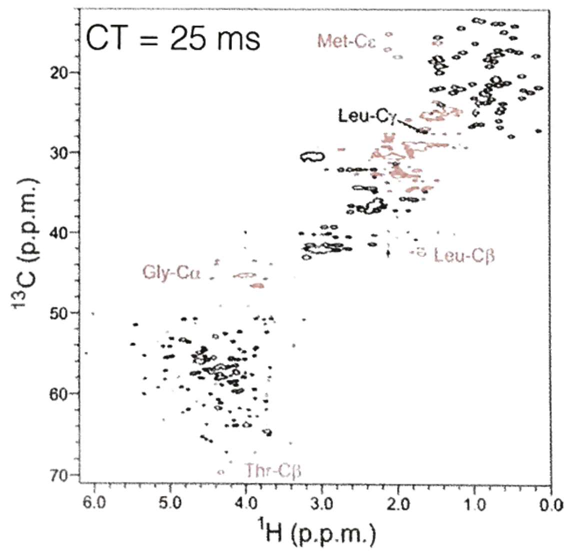
- Almost no decay in FID of CT-HSQC — but signal much weaker to begin with!
- Linear prediction particularly effective
- Mirror image LP can be used to double size of input data

Linear prediction



Extend FID by fitting autoregression model to predict future points – can reduce truncation artifacts

Constant time acquisition



$2T = n / {}^{1}J_{CC}$

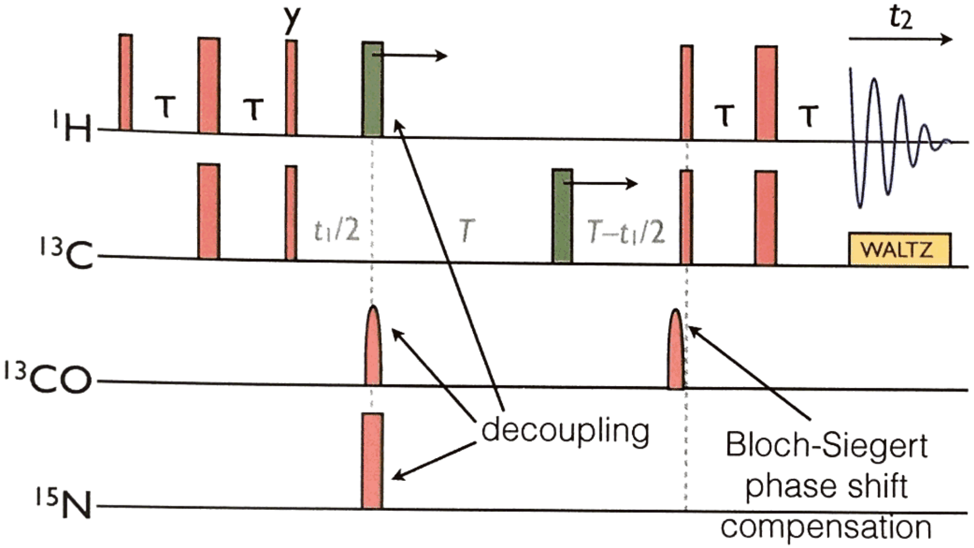
With n=1, sign of peak depends on number of directly bonded ^{13}C atoms

Useful for identification of e.g. Met Cε

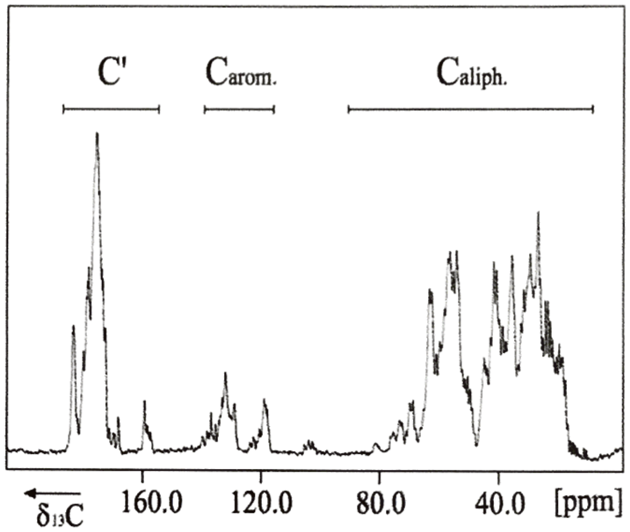
$\cos(2\pi J T) \rightarrow -1$

except Met ($J=0$) $\rightarrow +1$

A complete CT-HSQC pulse sequence

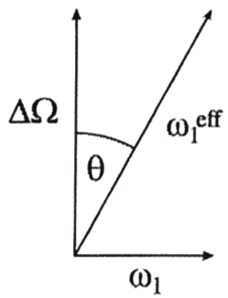


Selective pulses (for ^{13}C)



Selective pulses (for ^{13}C)

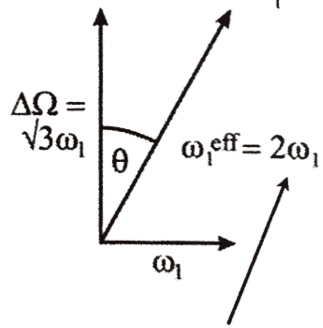
- How to achieve selective excitation/inversion of aliphatics (40 ppm) leaving carbonyls (170 ppm) untouched?
- Pulsing with field strength ω_1 on C_{aliph} will create an effective field ω_{eff} on C' (at offset $\Delta\Omega$ from C_{aliph})
- Trick: choose ω_{eff} such that 90° or 180° rotation of C_{aliph} causes 360° rotation of C'



Selective pulses

$\omega_{eff}^2 = \omega_1^2 + \Delta\Omega^2 = (2\omega_1)^2$
$\Rightarrow \omega_1 = \Delta\Omega/\sqrt{3}$

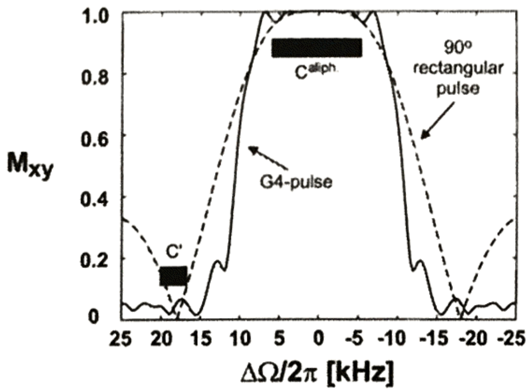
- For 90° pulse:
  $\omega_1 = \Delta\Omega / \sqrt{15}$
- For 180° pulse:
  $\omega_1 = \Delta\Omega / \sqrt{3}$
- Remember:
  $\omega_1 = 1 / (2\pi\, t_{360°})$
- So choose pulse lengths:
  $t_{90°} = \sqrt{15} / (4\, \Delta v)$
  $t_{180°} = \sqrt{3} / (2\, \Delta v)$
  and calibrate power accordingly



For 360° rotation off-resonance with 180° rotation on-resonance

Selective ^{13}C excitation – example

- 700 MHz ^{1}H
  176 MHz ^{13}C
- C_{aliph} ~ 50 ppm
  C' ~ 175 ppm
- $\Delta v = (175 - 50) \times 176$
  $= 22$ kHz
- $t_{90°} = \sqrt{15} / (4\, \Delta v)$
  $= 44.0\ \mu s$

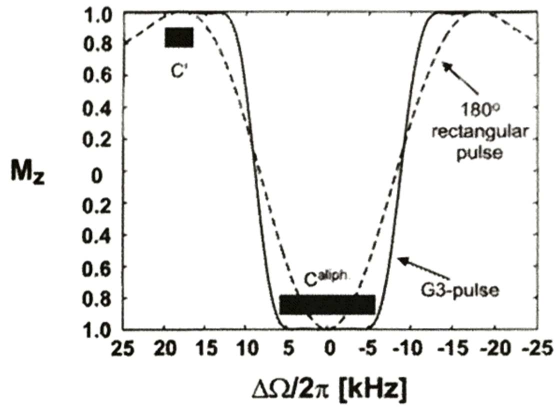


Compare excitation profile with 400 μs selective pulse

Trade off quality of frequency selection for pulse length

Selective ^{13}C inversion – example

- 700 MHz ^{1}H
  176 MHz ^{13}C
- C_{aliph} ~ 50 ppm
  C' ~ 175 ppm
- $\Delta v = (175 - 50) \times 17$
  $= 22$ kHz
- $t_{90°} = \sqrt{3} / (2\, \Delta v)$
  $= 39.4\ \mu s$



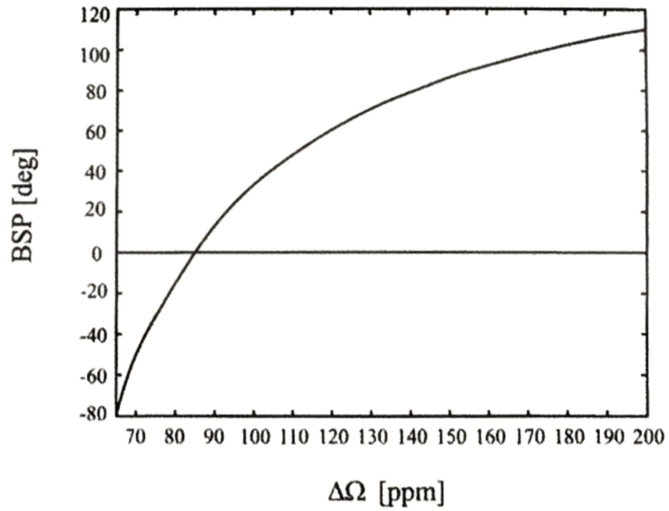
Compare inversion profile with 250 μs selective pulse

Notice that the 180° pulse is shorter and therefore needs higher power than 90° pulse!

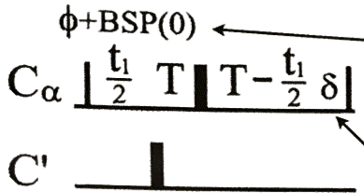
Bloch-Siegert shifts

- During a selective pulse (of length t_p) off-resonance spins undergo a 2π rotation and therefore experience no phase change (in the on-resonance rotating frame)
- However, in the absence of the pulse the same spins would experience a phase change:
  $$\Delta\phi = t_p\, \Delta\Omega$$
- Therefore, from the perspective of the off-resonance spins the selective pulse causes a phase shift:
  $$\Delta\phi = -t_p\, \Delta\Omega$$
- This is the '**Bloch-Siegert phase shift**'
- Also relevant for shaped pulses

Bloch-Siegert shifts

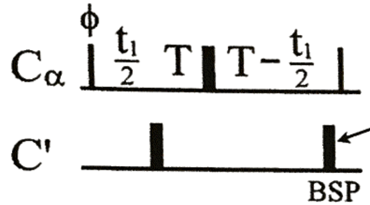


Compensating for Bloch-Siegert phase shifts



adjusting pulse phase corrects zero-order BSP

extra evolution delay compensates first-order BSP

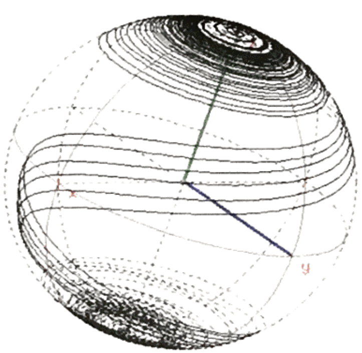


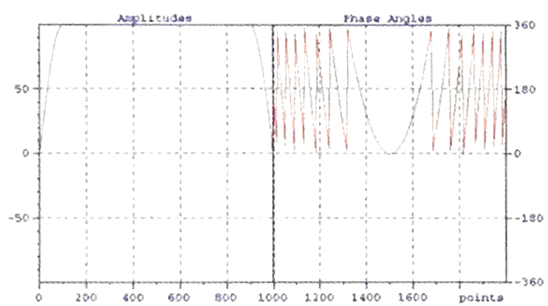
second identical shaped pulse compensates for all orders of BSP – but there's a loss in sensitivity associated with adding extra pulses

Adiabatic pulses

- Low-power pulses for [selective] excitation or inversion
- Insensitive to miscalibration
- Very wide bandwidth
- Operate on different principle to hard pulses or shaped pulses: slowly sweep field so that magnetisation vectors stay locked to B_{eff}
- Must satisfy adiabatic condition (slowly changing Hamiltonian): $\left|\frac{d\theta}{dt}\right| \ll \omega_{\text{eff}}$
- Disadvantage – long pulses, relaxation losses

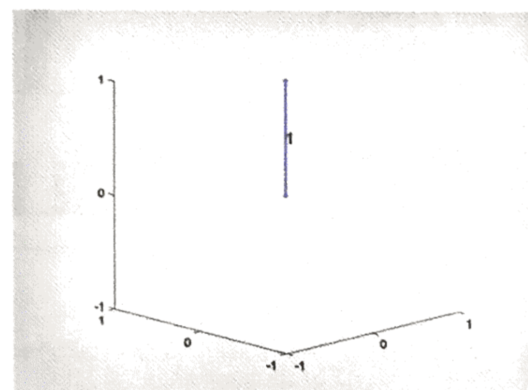
Adiabatic pulses





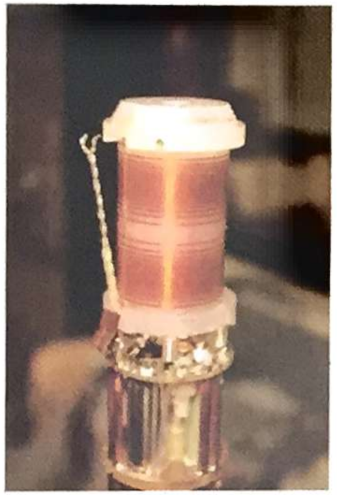
CHIRP pulse

Adiabatic pulses



tan/tanh pulse

NMR: pulsed-field gradients

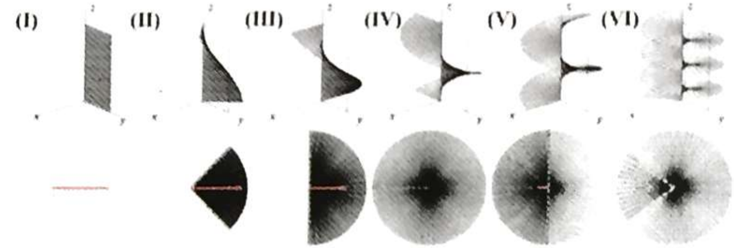


Disruption of field homogeneity

Magnetic field strength linearly proportional to position along z-axis:

$$B = B_0 + G \cdot z$$

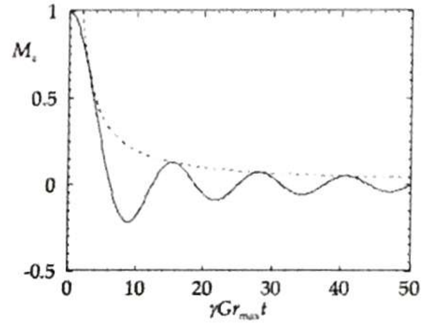
Uses of gradient pulses: purge pulses



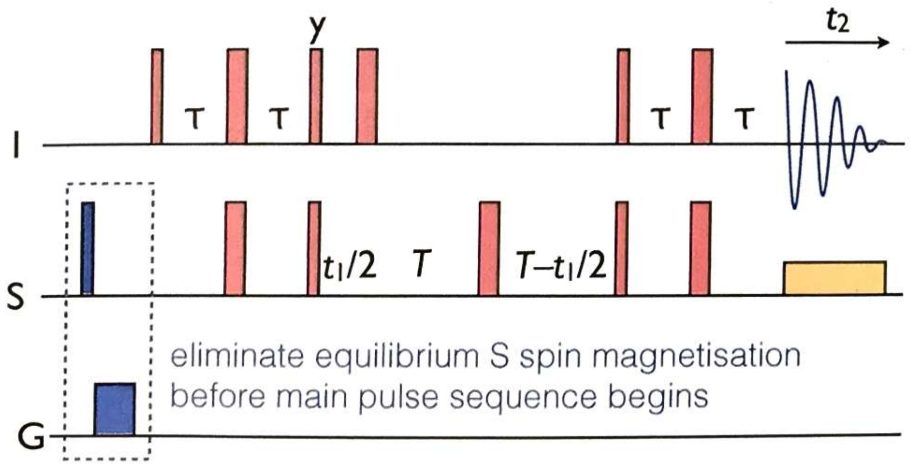
Phase from gradient applied for time *t*:

$$\phi = \gamma G t z$$

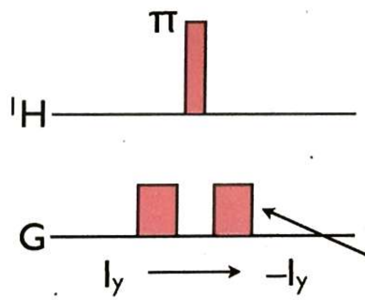
This results in rapid loss of signal when integrated over the length of the NMR tube



Uses of gradient pulses: purge pulses



Uses of gradient pulses: refocussing pulses



Important role of gradients is eliminating imperfections in 180° pulses

Must distinguish between refocussing and inversion pulses

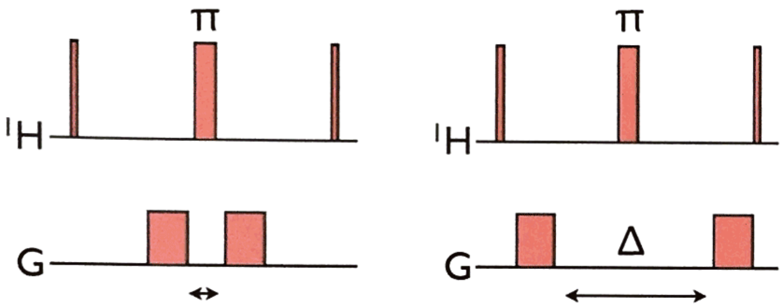
Only spins flipped exactly 180° in transverse plane will be refocussed by second gradient

$$I_y \xrightarrow{\text{gradient}} I_y \cos(\gamma G t z) - I_x \sin(\gamma G t z)$$

$$\xrightarrow{\pi I_x} -I_y \cos(\gamma G t z) - I_x \sin(\gamma G t z)$$

$$\xrightarrow{\text{gradient}} \begin{matrix} -I_y \cos^2(\gamma G t z) + I_x \cos(\gamma G t z)\sin(\gamma G t z) \\ -I_x \sin(\gamma G t z)\cos(\gamma G t z) - I_y \sin^2(\gamma G t z) \end{matrix} = -I_y$$

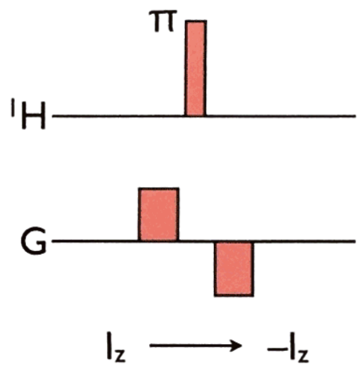
Placement of refocussing gradients



Long delays between gradient pairs will result in lower signal intensity due to diffusion

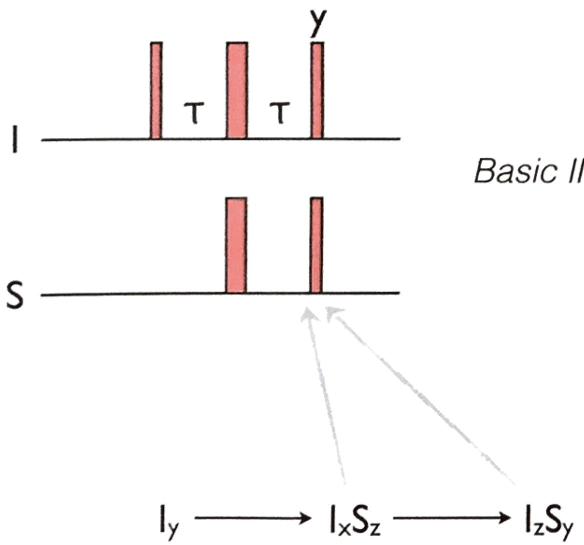
$$\frac{I}{I_0} = \exp\left[-(G\gamma\delta)^2(\Delta - \delta/3)D\right]$$

Uses of gradient pulses: inversion pulses



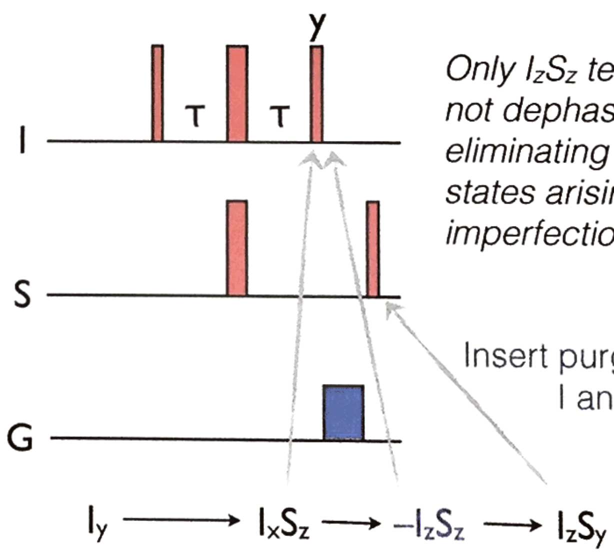
- Only longitudinal magnetisation survives pair of purge gradients
- Sign change of second gradient gives opposite effect to refocussing element – maximum suppression of transverse magnetisation

Uses of gradient pulses: zz filter



Basic INEPT transfer…

Uses of gradient pulses: zz filter

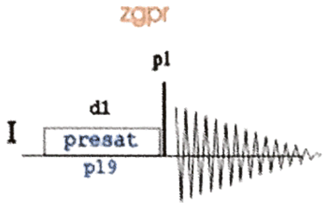


Only I_zS_z term (and Iz or S_z) is not dephased by gradient – eliminating undesirable spin states arising from pulse imperfections

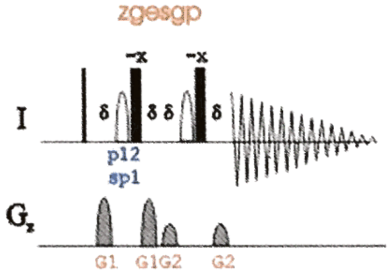
Insert purge gradient between I and S π/2 pulses

Solvent suppression

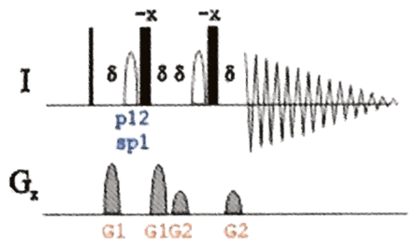
- Pre-saturation (zgpr)
  – selective saturation of H_2O resonance
  – saturation can transfer to exchangable protons



- Excitation sculpting (zgesgp)
  – elimination using selective excitation of H_2O and purge gradients



Water suppression – excitation sculpting



- Selective excitation of H_2O during spin-echo ensures H_2O is not rephased by gradient pair
- Selective excitation has better frequency selectivity than selective inversion
- Use of two gradient pairs ensures excellent water suppression
- Water returned to +z
- Water is dephased but not saturated
- No saturation of exchangable protons or saturation transfer – better sensitivity

Water suppression – excitation sculpting

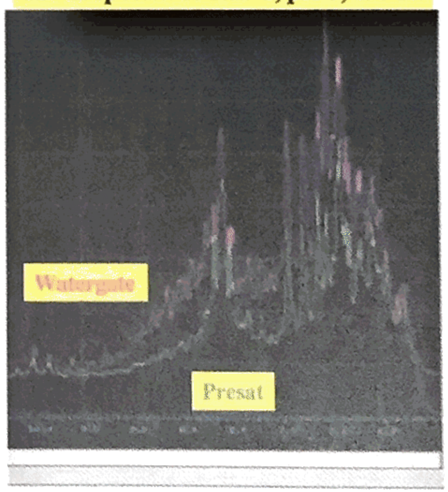
tilde indicates gradient encoded magnetisation

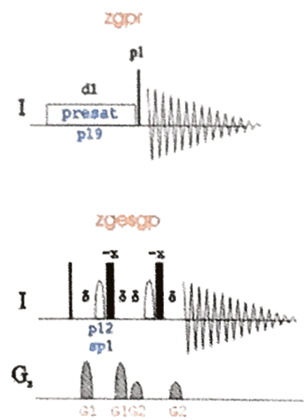
$$I_z \xrightarrow{(\pi/2)\, I_x} -I_y \xrightarrow{G_1} \widetilde{-I_y} \dashrightarrow \xrightarrow{\pi\, I_{-x}} \widetilde{I_y} \xrightarrow{G_1} -I_y \xrightarrow{\text{repeat } (G_2)} I_y$$

$$I_z \xrightarrow{(\pi/2)\, I_x} -I_y \xrightarrow{G_1} \widetilde{-I_y} \xrightarrow{(\pi/2)\, \text{water}_x} \widetilde{I_z} \xrightarrow{\pi\, I_{-x}} \widetilde{-I_z} \xrightarrow{G_1} \widetilde{-I_z} \xrightarrow{\text{repeat } (G_2)} \approx I_z$$

Solvent suppression

1D ^1H spectra of TEP-I, pH 6, 290 K.

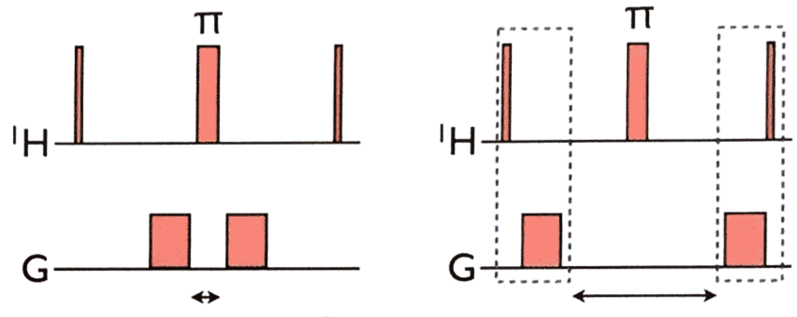




Radiation damping

- 700 MHz (on resonance) rf pulse in probe induces 90° rotation of spins
- Spins precess at Larmor frequency (700 MHz)
- Changing magnetic field of spins induces 700 MHz rf signal in probe ('the signal')
- BUT! 700 MHz rf signal in probe induces rotation of spins…
- Result: rapid rotation of H_2O back to equilibrium (taking other spins along for the journey)
- Practical significance: must take precautions in pulse sequence if placing H_2O in xy plane or on –z

Placement of refocussing gradients



Immediate dephasing of H_2O placed in *xy* plane prevents radiation damping – keep gradients close to 90° pulses!

Long delays between gradient pairs will result in lower signal intensity due to diffusion

Water suppression: HSQC example