Combinations of three spins

$$\text{Spin } \tfrac{1}{2} + \text{spin } \tfrac{1}{2} \begin{cases} \text{Spin } 0 \text{ (singlet, antisym)} & \xrightarrow{+\text{spin } \frac{1}{2}} \text{spin } \tfrac{1}{2} \\ \text{Spin } 1 \text{ (triplet, symmetric)} & \xrightarrow{+\text{spin } \frac{1}{2}} \begin{array}{l} \text{spin } \tfrac{1}{2} \\ \text{spin } \tfrac{3}{2} \end{array} \end{cases}$$
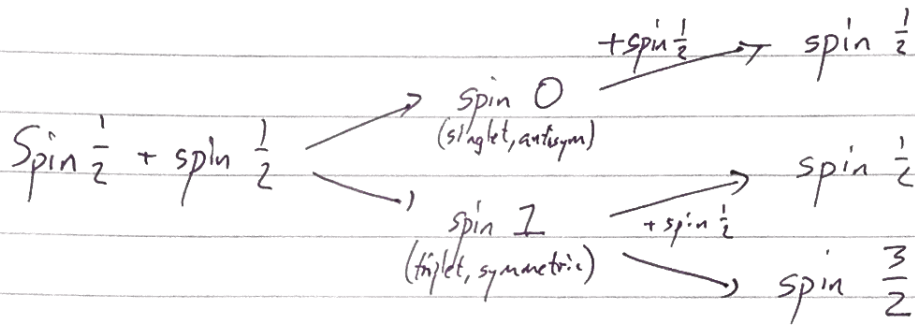
$$\tfrac{1}{2} \otimes \tfrac{1}{2} = 0 \oplus 1$$

$$\tfrac{1}{2} \otimes \tfrac{1}{2} \otimes \tfrac{1}{2} = \left(0 \oplus 1\right) \otimes \tfrac{1}{2} = \left(0 \otimes \tfrac{1}{2}\right) \oplus \left(1 \otimes \tfrac{1}{2}\right)$$

$$= \tfrac{1}{2} \oplus \tfrac{1}{2} \oplus \tfrac{3}{2}$$

$\longrightarrow$ 3 manifolds with distinct symmetry properties.

Choice of basis set for analysing methyl groups:

C and H spins weakly coupled $\rightarrow$ tensor product

$$|\psi_{CH}\rangle = |\psi_C\rangle|\psi_H\rangle$$

$\{|\alpha\rangle, |\beta\rangle\}$

$\{|\alpha\alpha\alpha\rangle, |\alpha\alpha\beta\rangle, |\alpha\beta\alpha\rangle, |\beta\alpha\alpha\rangle,$
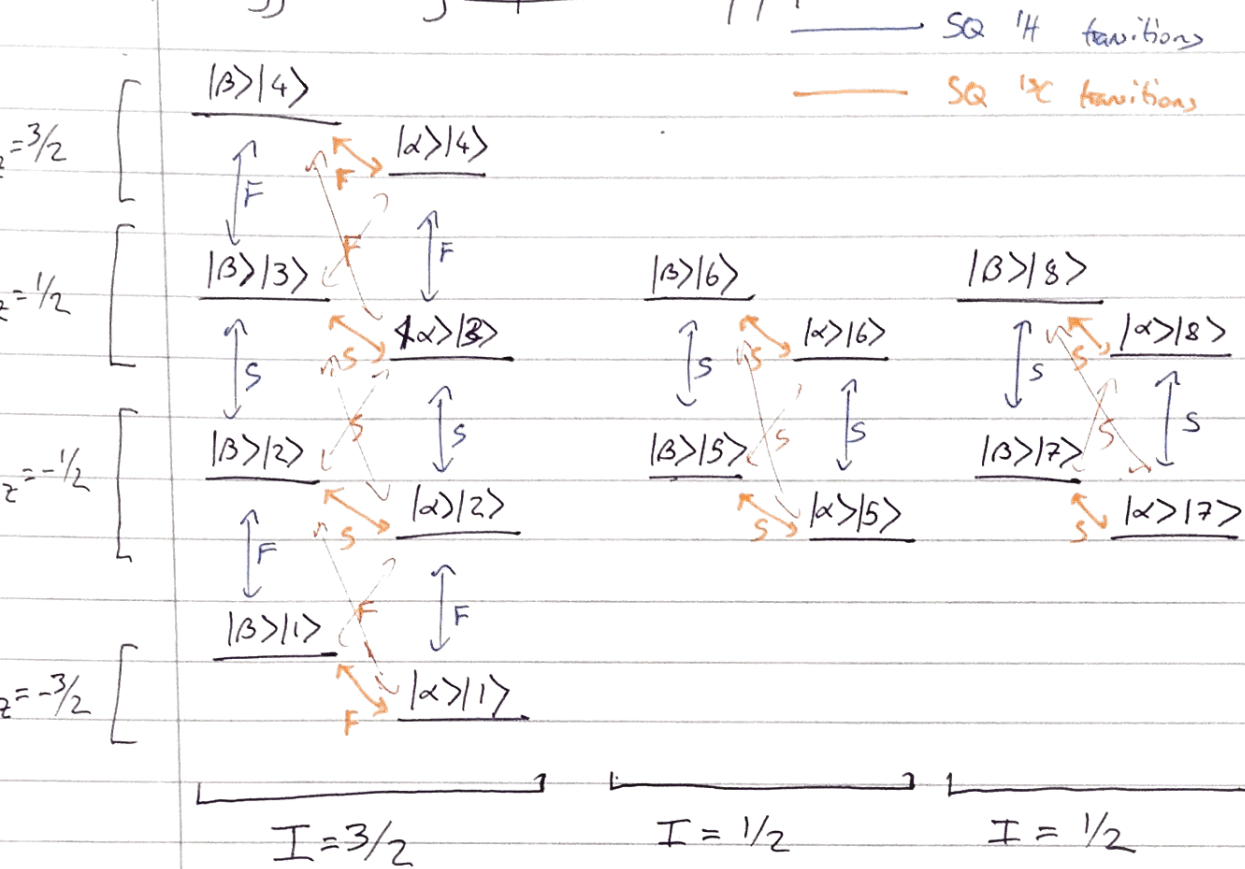$|\beta\alpha\beta\rangle, |\beta\beta\alpha\rangle, |\alpha\beta\beta\rangle, |\beta\beta\beta\rangle\}$

form **symmetrised** combinations.

	total spin, I	exchange 2/3 symmetry
$\|1\rangle = \|\alpha\alpha\alpha\rangle$	3/2	S
$\|2\rangle = (\|\alpha\alpha\beta\rangle + \|\alpha\beta\alpha\rangle + \|\beta\alpha\alpha\rangle)/\sqrt{3}$	3/2	S
$\|3\rangle = (\|\beta\beta\alpha\rangle + \|\beta\alpha\beta\rangle + \|\alpha\beta\beta\rangle)/\sqrt{3}$	3/2	S
$\|4\rangle = \|\beta\beta\beta\rangle$	3/2	S
$\|5\rangle = (\|\alpha\alpha\beta\rangle - \|\alpha\beta\alpha\rangle)/\sqrt{2}$	1/2	A
$\|6\rangle = (\|\beta\alpha\beta\rangle - \|\beta\beta\alpha\rangle)/\sqrt{2}$	1/2	A
$\|7\rangle = (\|\alpha\alpha\beta\rangle + \|\alpha\beta\alpha\rangle - 2\|\beta\alpha\alpha\rangle)/\sqrt{6}$	1/2	S
$\|8\rangle = (\|\beta\beta\alpha\rangle + \|\beta\alpha\beta\rangle - 2\|\alpha\beta\beta\rangle)/\sqrt{6}$	1/2	S

$\rightarrow$ Division into 3 manifolds with distinct symmetries.

These form subspaces that evolve independently, during free evolution but also through pulses etc.
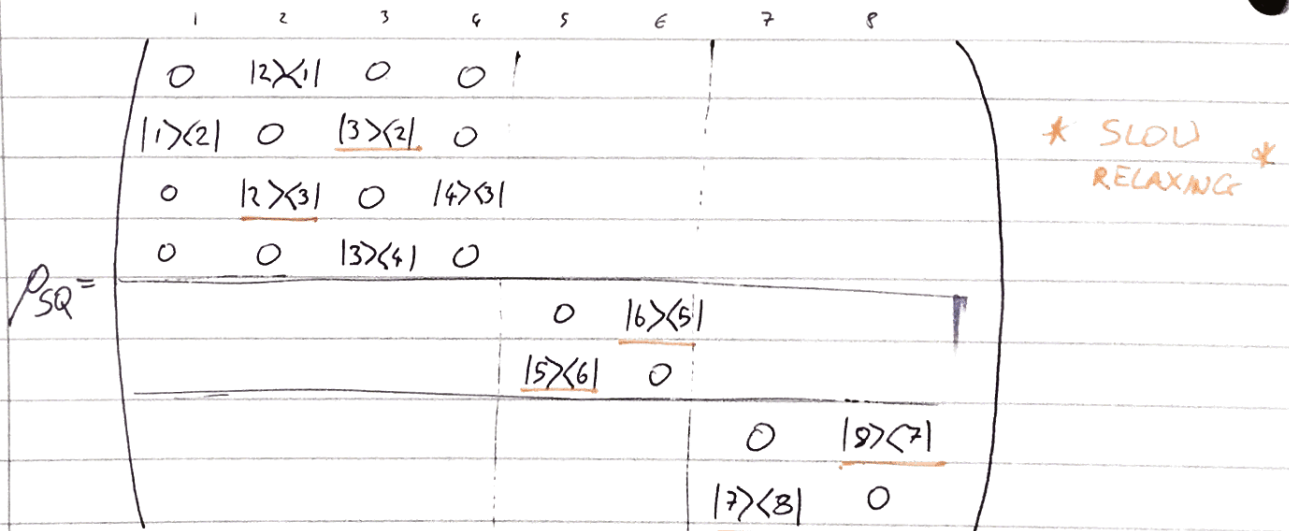
Energy level diagram for isolated methyl group

——— SQ ¹H transitions → $SQ\ ^1H$ transitions
——— SQ ¹³C transitions → $SQ\ ^{13}C$ transitions

$z = \tfrac{3}{2}$: $|\beta\rangle|4\rangle$ $|\alpha\rangle|4\rangle$

$z = \tfrac{1}{2}$: $|\beta\rangle|3\rangle$ $|\alpha\rangle|3\rangle$ $|\beta\rangle|6\rangle$ $|\alpha\rangle|6\rangle$ $|\beta\rangle|8\rangle$ $|\alpha\rangle|8\rangle$

$z = -\tfrac{1}{2}$: $|\beta\rangle|2\rangle$ $|\alpha\rangle|2\rangle$ $|\beta\rangle|5\rangle$ $|\alpha\rangle|5\rangle$ $|\beta\rangle|7\rangle$ $|\alpha\rangle|7\rangle$

$z = -\tfrac{3}{2}$: $|\beta\rangle|1\rangle$ $|\alpha\rangle|1\rangle$

(transitions labelled F and S)

$I = 3/2$ $I = 1/2$ $I = 1/2$

What transitions are allowed?

10 × ¹H SQ transitions split into 1:1 doublet

8 × ¹³C SQ transition split into 1:3:3:1 quartet

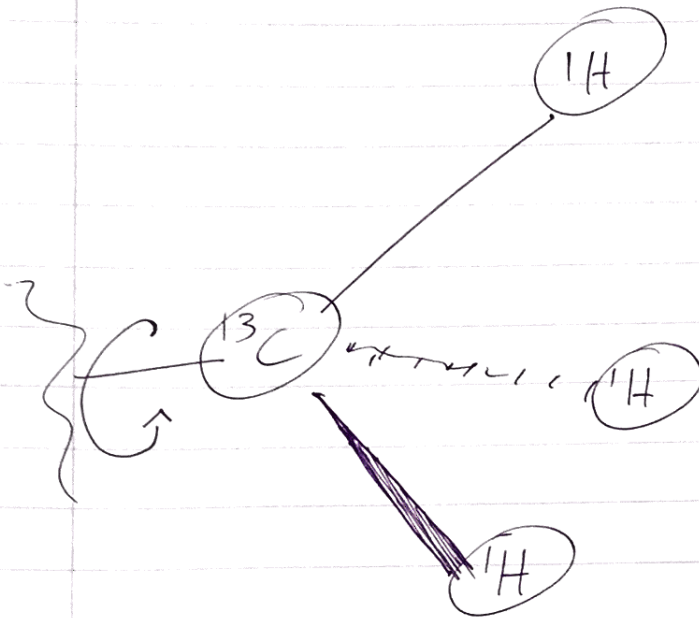'Preview' of methyl TROSY effect : SQ coherences have different relaxation rates:

$$
\rho_{SQ} = \begin{pmatrix}
0 & |2\rangle\langle1| & 0 & 0 & & & & \\
|1\rangle\langle2| & 0 & |3\rangle\langle2| & 0 & & & & \\
0 & |2\rangle\langle3| & 0 & |4\rangle\langle3| & & & & \\
0 & 0 & |3\rangle\langle4| & 0 & & & & \\
 & & & & 0 & |6\rangle\langle5| & & \\
 & & & & |5\rangle\langle6| & 0 & & \\
 & & & & & & 0 & |8\rangle\langle7| \\
 & & & & & & |7\rangle\langle8| & 0
\end{pmatrix}
$$

(columns labelled 1 2 3 4 5 6 7 8)

★ SLOW RELAXING ★

Next stage —

① calculating relaxation rates

② pulse sequence design

③ labelling schemes

Methyl relaxation:

Sources of relaxation:
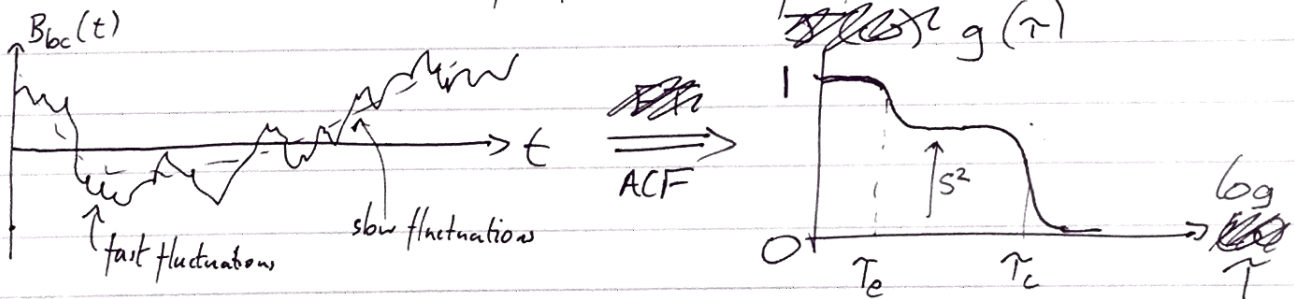
- dipolar $\underset{\searrow C-H}{\overset{\nearrow H-H}{\longleftarrow}}$ ----→ external

- CSA - not significant
- exchange, paramagnets etc.
- external field
  (i.e. other protons)

From now on, going to assume we are in MACROMOLECULAR LIMIT: $\omega_0 \tau_c \gg 1$

In this limit, we only care about $J(0)$.

Relaxation is caused by fluctuating fields:

'Model-free formalism'

Relaxation rates related to field strength and reduced spectral density:

$$R = a \cdot \overline{B_{loc}^2} \cdot \overline{J}(0) + b \cdot \overline{B_{loc}^2} \cdot \overline{J}(\omega_c) + c \cdot \overline{B_{loc}^2} \cdot \overline{J}(\omega_H) + \dots$$

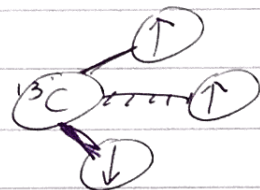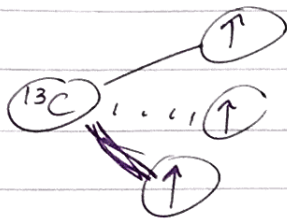reduced spectral density $\quad j(\omega) = 2 \int_0^\infty e^{-i\omega\tau} g(\tau) \, d\tau$

Macromolecular limit: $R = a \cdot \overline{B_{loc}^2} \cdot j(0)$

$$j(0) = 2 \int_0^\infty g(\tau) \, d\tau \approx S^2 \tau_c + (1-S^2)\tau_e \approx S^2 \tau_c$$

^{13}C relaxation rate:

$$R_{2,C} \propto \overline{B_{loc}^2} \, j(0) \sim \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_C^2 \gamma_H^2}{r_{CH}^6} \cdot S^2 \tau_C$$

Effect of H spin states?

$|\alpha\alpha\rangle : B_{loc} \propto 3\gamma_H$
$|\beta\beta\rangle$

$|\alpha\alpha\beta\rangle : B_{loc} \propto \gamma_H$
$|\alpha\beta\beta\rangle$

9× difference in relaxation rates.

$\{$ cancellation of dipolar fields $\}$
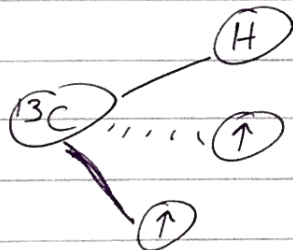
So we expect ^{13}C to have 2 relaxation rates!

$$R_{2,C}^{fast} = \frac{1}{5} \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_C^2 \gamma_H^2}{r_{CH}^6} S^2 \tau_C \qquad |\alpha\alpha\rangle, |\beta\beta\rangle$$

and $$R_{2,C}^{slow} \sim \frac{1}{45} \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_C^2 \gamma_H^2}{r_{CH}^6} \cdot S^2 \tau_C \qquad |\alpha\alpha\beta\rangle, |\alpha\beta\beta\rangle$$

N.B. unlike NH TROSY, this is <u>field strength independent</u>.
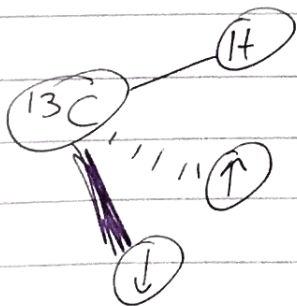
^1H relaxation rate:

2 sources: C-H dipole and H-H dipoles.

$$R_{2,H}^{(fast)} = \frac{1}{6}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_C^2 \gamma_H^2}{r_{CH}^6} S^2 \tau_C + \frac{9}{20}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_H^4}{r_{CH}^6} \cdot S^2 \tau_C$$

$$\underbrace{|\alpha\alpha\alpha\rangle\langle\alpha\alpha\beta|}_{\text{like} \quad \text{like}} \quad \text{and} \quad |\beta\beta\beta\rangle\langle\beta\beta\alpha| \quad + \text{symmetrise}$$

HH transition

cancellation!

$$R_{2,H}^{slow} = \frac{1}{6}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_C^2 \gamma_H^2}{r_{CH}^6} \cdot S^2 \tau_C$$

unlike

$$\cancel{|\alpha\alpha\beta\rangle\langle\alpha} \quad |\beta\alpha\alpha\rangle\langle\beta\alpha\beta|$$

transition

Evolution of density operators

How does a density operator $\hat{\rho}$ evolve in time, under either the Hamiltonian for free evolution, $\hat{H}_{free}$, or for an r.f. pulse, $\hat{H}_{pulse}$?

The time evolution is given by the Von Neumann equation:

$$\frac{\partial \rho}{\partial t} = \frac{1}{i\hbar}(H\rho - \rho H) = \frac{1}{i\hbar}[H, \rho]$$

This is easy to derive from the TDSE:

$$i\hbar \frac{\partial}{\partial t}|\Psi\rangle = H|\Psi\rangle, \quad h.c. \quad -i\hbar\frac{\partial}{\partial t}\langle\Psi| = \langle\Psi|H$$

The density operator is defined as $\rho = \sum p_i |\Psi_i\rangle\langle\Psi_i|$

$$\therefore \frac{\partial \rho}{\partial t} = \sum p_i \left[\frac{\partial}{\partial t}\left(|\Psi_i\rangle\langle\Psi_i|\right)\right] = \sum p_i \left[\left(\frac{\partial}{\partial t}|\Psi_i\rangle\right)\langle\Psi_i| + |\Psi_i\rangle \frac{\partial}{\partial t}\langle\Psi_i|\right]$$

$$= \sum p_i \left[\frac{1}{i\hbar}H|\Psi_i\rangle\langle\Psi_i| + |\Psi_i\rangle\left(-\frac{1}{i\hbar}\right)\langle\Psi_i|H\right]$$

$$= \frac{1}{i\hbar}\left[H\left(\sum_{p_i}|\Psi_i\rangle\langle\Psi_i|\right) - \left(\sum_{p_i}|\Psi_i\rangle\langle\Psi_i|\right)H\right]$$

$$= \frac{1}{i\hbar}(H\rho - \rho H) \quad .$$
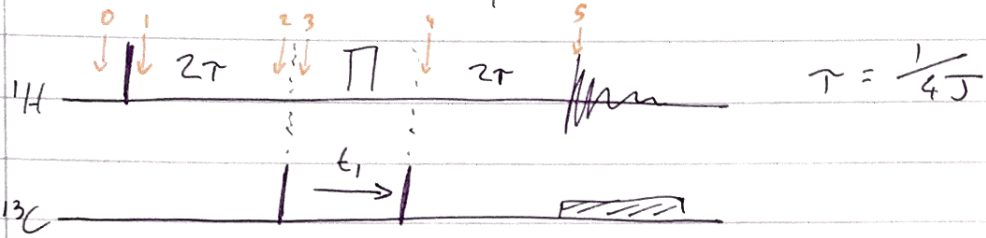
What is the solution of the Von Neumann equation? If H is time independent, easy to solve the TDSE:

$$\frac{\partial}{\partial t}|\Psi\rangle = \frac{1}{i\hbar}H|\Psi\rangle \quad \text{and} \quad \frac{\partial}{\partial t}\langle\Psi| = \frac{1}{i\hbar}\langle\Psi|H$$

$$\Rightarrow |\Psi(t)\rangle = \exp\left(\frac{1}{i\hbar}Ht\right)|\Psi(0)\rangle \quad \text{and} \quad \langle\Psi(t)| = \langle\Psi(0)|\exp\left(-\frac{1}{i\hbar}Ht\right)$$

$$\therefore \rho(t) = \sum p_i |\Psi_i(t)\rangle\langle\Psi_i(t)|$$

$$= \sum p_i e^{-iHt/\hbar}|\Psi(0)\rangle\langle\Psi_i(0)|e^{+iHt/\hbar} = e^{-iHt/\hbar}\left(\sum p_i|\Psi_i\rangle\langle\Psi_i|\right)e^{iHt/\hbar}$$

$$= e^{-iHt/\hbar}\rho(0)e^{+iHt/\hbar} \quad .$$

Analysis of HMQC sequence

^1H

2τ Π 2τ

^{13}C

t_1

$\tau = \frac{1}{4J}$

Initial magnetisation:

$$H_z = \frac{1}{2}\left(3\,|1\rangle\langle1| + |2\rangle\langle2| - |3\rangle\langle3| - 3|4\rangle\langle4| + |5\rangle\langle5| - |6\rangle\langle6| + |7\rangle\langle7| + |8\rangle\langle8|\right)$$

↑ coefficients are simply the energy ↑ levels

(recall that at equilibrium $\rho_{eq} = \frac{1}{Z}e^{-H_z/kT} \approx \frac{1}{kT\cdot Z}(I - H_z)$,

ie. H_z is <u>difference</u> in pop'ns relative to identity matrix)

Effect of $\frac{\pi}{2}H_x$ pulse:

→ mixes $|1-4\rangle$ together

$$e^{-i\left(\frac{\pi}{2}\right)H_x} = \begin{pmatrix} 4\times4 & & O \\ \hline & 2\times2 & O \\ \hline O & O & 2\times2 \end{pmatrix}$$

→ mixes $|5\rangle$ and $|6\rangle$ together

→ mixes $|7\rangle$ and $|8\rangle$ together

$$\therefore \rho_1 = e^{-i\left(\frac{\pi}{2}\right)H_x}\,\rho_0\,e^{i\left(\frac{\pi}{2}\right)H_x}$$

$$= \frac{i\sqrt{3}}{2}\left(|1\rangle\langle2| - |2\rangle\langle1| + |3\rangle\langle4| - |4\rangle\langle3|\right) + i\left(|2\rangle\langle3| - |3\rangle\langle2|\right)$$

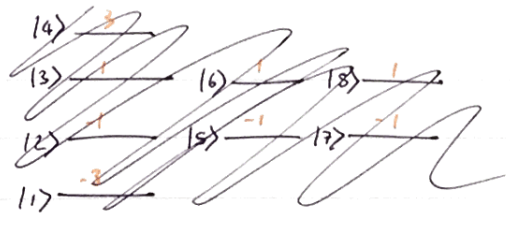$$+ \frac{i}{2}\left(|5\rangle\langle6| - |6\rangle\langle5| - |7\rangle\langle8| + |8\rangle\langle7|\right)$$

Evolution for 2τ under $2H_zC_z$ $e^{-i(2\pi J H_zC_z)/2J} = e^{-i\pi H_zC_z}$

$$\rho_2 = e^{-i\pi H_zC_z}\,\rho_1\,e^{i\pi H_zC_z} \qquad \boxed{12} = |1\rangle\langle2|$$

$$= 2C_z\left[\frac{\sqrt{3}}{2}e^{2\tau R_{2H}^\dagger}\left(\boxed{12} + \boxed{21} + \boxed{34} + \boxed{43}\right) + e^{-2\tau R_{2H}^s}\left(\boxed{23} + \boxed{32}\right) + \frac{i}{2}e^{2\tau R_{2H}^s}\left(\boxed{56} + \boxed{65} + \boxed{78} + \boxed{87}\right)\right]$$

$$\rho_5 = i\cos(\omega_c t_1)\left[\frac{\sqrt{3}}{2}L_H^{2\tau,f}\,L_{CH}^{t_1,f}\,L_H^{2\tau,f}\left(\boxed{12} - \boxed{21} + \boxed{34} - \boxed{43}\right)\right.$$

$$\left. + \frac{1}{2}L_H^{2\tau,s}\,L_{CH}^{t_1,s}\,L_H^{2\tau,s}\left(2\boxed{23} - 2\boxed{32} + \boxed{56} - \boxed{65} - \boxed{78} + \boxed{87}\right)\right]$$

Observable magnetisation: $H_- = H_x - iH_y$

$$\text{Signal} = \text{Tr}\left(\rho \cdot H_-^\dagger\right)$$

$$= 6\cos(\omega_c t_1)\left[\cancel{\exp}\, e^{-(4\tau+t_2)R_{2H}^f - t_1 R_{2CH}^f} + e^{-(4\tau+t_2)R_{2H}^s - t_1 R_{2CH}^s}\right]$$

50% of signal is retained when $R_2^f \gg R_2^s$

Comparison with HSQC sequence

$$^1H \quad |\tau \sqcap \tau| \quad \sqcap \quad |\tau \sqcap \tau| \text{\tiny mmm}$$

$$^{13}C \quad \sqcap \quad \xrightarrow{t_1} \quad | \sqcap \quad \boxed{\text{////}}$$
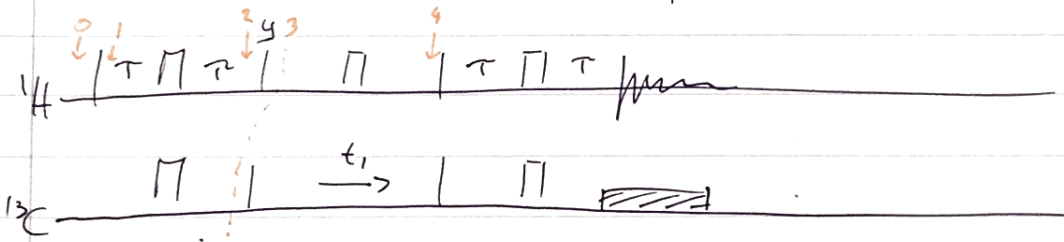
Up to end of first spin-echo (ρ_2), ~~sequence in~~ density matrix is identical to HMQC.

$$\rho_2 = 2C_z\left[e^{-2\tau R_{2H}^f}\left(\textcircled{13} + \textcircled{21} + \textcircled{34} + \textcircled{43}\right) + e^{-2\tau R_{2H}^s}\left(\textcircled{23} + \textcircled{32} + \tfrac{1}{2}\left[\textcircled{56} + \textcircled{65} - \textcircled{78} - \textcircled{87}\right]\right)\right]$$

$$\rho_3 = e^{-i\frac{\pi}{2}H_y}\,\rho_2\,e^{i\frac{\pi}{2}H_y}$$

slow and fast relaxing coherences mixed together

$$= 2C_z\left[\tfrac{3}{4}\left(e^{-2\tau R_{2H}^f} + e^{-2\tau R_{2H}^s}\right)\left(\textcircled{11} - \textcircled{44}\right) + \tfrac{1}{4}\left(3e^{-2\tau R_{2H}^f} - e^{-2\tau R_{2H}^s}\right)\left(\textcircled{22} - \textcircled{33}\right)\right.$$

$$\left. + \tfrac{1}{2}e^{-2\tau R_{2H}^s}\left(\textcircled{55} + \textcircled{66} + \textcircled{77} - \textcircled{88}\right)\right]$$

· Subsequent pulses ~~will~~ will continue to mix F/S coherences together

· (Almost) Only spin-$\frac{1}{2}$ manifolds survive to give final signal !

19% retained when $R_2^f \gg R_2^s$

cf. 50% for HMQC $\longrightarrow$ 2·5× more sensitive.

How much signal arises from spin-$\frac{1}{2}$ manifolds?
Consider a simple pulse-observe experiment:

^1H —————————— ρ_1 identical to previous calculations.

^{13}C ——————————

Observed signal $= \text{Tr}\left(\rho \cdot H_+\right)$

$$= \text{Tr } i \begin{pmatrix} 3/2 & & & & & \\ & 2 & & & & \\ -\sqrt{3} & & 3/2 & & & \\ & -\sqrt{3} & & 0 & & \\ & & & & \frac{1}{2} & \\ & & & & & 0 \\ & & & & & & \frac{1}{2} \\ & & & & & & & 0 \end{pmatrix}$$

not observed

$$= 6i$$

of which only $2 \times \frac{1}{2} i$ arises from spin $\frac{1}{2}$ manifolds.

$\Rightarrow \frac{1}{6}$ of total signal $= \underline{\underline{17\%}}$.