Supporting Information

A Methyl ^{1}H Double Quantum CPMG Experiment to Study Protein Conformational Dynamics

Bloch-McConnell equations considering the complete spin 3/2 manifold

In the main text we have presented the Bloch-McConnell equations describing the evolution of DQ coherences during the course of the CPMG pulse-element, Eq. (2). In general, such a description is sufficient to accurately fit the resulting methyl ^{1}H DQ CPMG dispersion curves. However, this simplified treatment cannot properly take into account evolution due to relaxation during the CPMG refocusing pulses. Here we consider the complete 3/2 manifold and include all of the density elements to show that differential relaxation effects of the coherences created during the refocusing pulses can lead to 'non-flat' profiles in the case of composite pulses. In this extensive calculation all of the density elements $|i\rangle\langle j|$ are considered, with the 4 relevant values of $|i\rangle$ (those associated with the 3/2 manifold) given in Figure 1. The evolution of the different coherences can be conveniently expressed as (Bain and Berno, 2011; Ghose, 2000)

$$\frac{d\vec{v}}{dt} = -\left(i\hat{E}_{FP} + i\hat{E}_{B_1} + \hat{R} + \hat{K}\right)\vec{v} \tag{S1}$$

where vector $\vec{v}$ consists of 64 elements, 16 arising from terms that are in-phase (IP) with respect to the methyl ^{13}C nucleus, and given by the expectation values of $|i\rangle\langle j|$ and 16 arising from the anti-phase (AP) terms, $2|i\rangle\langle j|C_z$. Finally, the number of terms is doubled because we consider those for both states *G* and *E* in the 2-site exchanging system. Thus $\vec{v}$ is given by

$$\vec{v} = [M^G_{11,IP}\ M^G_{12,IP}\ M^G_{13,IP}\ M^G_{14,IP}\ M^G_{21,IP}\ \dots\ M^G_{44,IP}\ M^G_{11,AP}\ M^G_{12,AP}\ \dots\ M^G_{44,AP}\ M^E_{11,IP}\ M^E_{12,IP}\ \dots\ M^E_{44,AP}]^{\mathrm{T}} \tag{S2}$$

where the superscript 'T' denotes transpose. Here $M^m_{ij,l}$ is the expectation value of $|i\rangle\langle j|$ (*l*=IP) or $2|i\rangle\langle j|C_z$ (*l*=AP) for $m \in \{G, E\}$. Matrices $\hat{E}_{FP}$, $\hat{E}_{B_1}$, $\hat{R}$ and $\hat{K}$ describe the evolution of the density elements due to free precession, the presence of B_1 fields (pulsing), from relaxation, and due to chemical exchange, respectively. Elements of $\hat{E}_{FP}$ were calculated, including both chemical shift and one-bond scalar couplings between ^{1}H-^{13}C spins (coupling constant of ^{1}J$_{\mathrm{CH}}$),

$$\hat{E}_{FP} = \begin{bmatrix} \Omega_G & \pi\,{}^1J_{CH} & 0 & 0 \\ \pi\,{}^1J_{CH} & \Omega_G & 0 & 0 \\ 0 & 0 & \Omega_E & \pi\,{}^1J_{CH} \\ 0 & 0 & \pi\,{}^1J_{CH} & \Omega_E \end{bmatrix} \otimes \begin{bmatrix} 0&0&0&0&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&1&0&0&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&2&0&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&0&3&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&0&0&-1&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&1&0&0&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&2&0&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&0&-2&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&0&0&-1&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&0&0&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&1&0&0&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&-3&0&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&0&-2&0&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&0&0&-1&0 \\ 0&0&0&0&0&0&0&0&0&0&0&0&0&0&0&0 \end{bmatrix} \tag{S3}$$

In Eq (S3) Ω_G (Ω_E) is the difference between the resonance frequency of a spin in state *G* (*E*) and the carrier, with both chemical shift and scalar coupled evolution scaling with coherence order.

Elements of $\hat{E}_{B_1}$ were calculated using a Hamiltonian of the form $2\pi B_1 \sum_{k=1}^{3} \hat{H}_x^k \cos\phi + \hat{H}_y^k \sin\phi$, that accounts for the effect of pulses on the three methyl protons, distinguished by superscript *k*. Here $\hat{H}_x^k$ and $\hat{H}_y^k$ are the operators for the *x* and *y* components of the spin angular momentum of methyl proton *k*, ϕ is the angle between the applied B_1 field (Hz) and the *x* axis in the rotating frame. $\hat{E}_{B_1}$ for state *G* ($\hat{E}_{B_1}^G$) is given by

$$\hat{E}_{B_1}^G = 2\pi B_1 \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \otimes$$

$$\begin{bmatrix}
0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & -e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{-i\phi} & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & 0 & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & -e^{i\phi} & 0 & 0 & e^{-i\phi} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -e^{-i\phi} & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & e^{-i\phi} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & e^{-i\phi} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & e^{i\phi} & 0 & 0 & 0 & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & -e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & e^{i\phi} & 0 & 0 & -e^{-i\phi} & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{-i\phi} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & 0 & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0 & -e^{i\phi} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -e^{-i\phi} & 0 & -\frac{\sqrt{3}}{2}e^{i\phi} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{\sqrt{3}}{2}e^{i\phi} & 0 & 0 & -\frac{\sqrt{3}}{2}e^{-i\phi} & 0
\end{bmatrix} \tag{S4}$$

Note that the tensor product with the identity matrix takes into account that there are both IP and AP terms (16 of each) for the density elements of state *G*. Finally, $\hat{E}_{B_1}$ is calculated according to

$$\hat{E}_{B_1} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \otimes \hat{E}_{B_1}^G \tag{S5}$$

Relaxation is taken into account by $\hat{R}$ that includes intra-methyl ^{1}H-^{13}C and ^{1}H-^{1}H dipolar contributions as well as the effects of external protons that have been modeled by considering a single additional proton that is placed at an effective distance from the methyl group in question so as to ‘account for all additional proton spins’, see below (Kay and Torchia, 1991; Tugarinov and Kay, 2007; Yuwen, Vallurupalli, and Kay, 2016). Only spectral density terms evaluated at 0 frequency, *j*(0), were retained, including both auto- and cross-correlation terms, as these are the dominant contributors to transverse relaxation in the macromolecular limit (Cavanagh et al., 2006; Tugarinov and Kay, 2007), except when calculating differences in rates between in-phase and anti-phase coherences where leading terms proportional to $j(\omega_C)$ were retained. The elements of $\hat{R}$ were obtained from Redfield theory (Cavanagh et al., 2006; Redfield, 1957) using the MathNMR package (Jerschow, 2005) or calculated using in-house written software. The first 16x16 elements of $\hat{R}$, corresponding to those for the in-phase elements of G, $\hat{R}_{IP}^{G}$, that, in turn, describe the relaxation of the first 16 elements of $\vec{v}$ are given by

$$\hat{R}_{IP}^{G}(j_{CH}, j_{HH}, j_{HH_E}) = \frac{1}{2}\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 4j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 9j_{CH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & j_{CH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 4j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 4j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & j_{CH} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & j_{CH}+9j_{HH} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 9j_{CH} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 4j_{CH}+9j_{HH} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & j_{CH}+9j_{HH} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

$$+\frac{j_{HH_E}}{8}\begin{bmatrix}
3 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 9 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 21 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 39 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 9 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 0 & 0 \\
-3 & 0 & 0 & 0 & 0 & 7 & 0 & 0 & 0 & 0 & -4 & 0 & 0 & 0 & 0 & 0 \\
0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 11 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 \\
0 & 0 & -3 & 0 & 0 & 0 & 0 & 21 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 21 & 0 & 0 & 0 & 0 & -3 & 0 & 0 \\
0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 11 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 \\
0 & 0 & 0 & 0 & 0 & -4 & 0 & 0 & 0 & 0 & 7 & 0 & 0 & 0 & 0 & -3 \\
0 & 0 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 9 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 39 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 21 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 9 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 3
\end{bmatrix} \tag{S6}$$

In Eq (S6)

$$j_{CH} = \left(\frac{\mu_o}{4\pi}\right)^2 \frac{2}{45}\frac{\gamma_H^2\gamma_C^2\hbar^2}{r_{HC}^6}\tau_C, \quad j_{HH} = \left(\frac{\mu_o}{4\pi}\right)^2 \frac{1}{10}\frac{\gamma_H^4\hbar^2}{r_{HH}^6}\tau_C, \quad j_{HH_E} = \left(\frac{\mu_o}{4\pi}\right)^2 \frac{2}{5}\frac{\gamma_H^4\hbar^2}{r_{HH_E}^6}\tau_C \tag{S7}$$

that includes intra-methyl ^{1}H-^{13}C (j_{CH}) and ^{1}H-^{1}H dipolar (j_{HH}) interactions, as well as contributions from a single external proton H$_E$ (j_{HH_E}). In Eq (S7) γ_p is the gyromagnetic ratio of spin p, r_{pq} is the distance between spins p and q, $\hbar$ is Planck's constant divided by 2π, τ_C is the assumed isotropic tumbling time and $S_2^{axis} = 1$. In deriving Eq (S7) we have assumed infinitely fast rotation of the methyl group about its symmetry axis. Note that in this limit both auto- and cross-spectral density terms that derive from intra-methyl dipolar interactions are equivalent (Kay and Torchia, 1991). We have chosen to model external protons by considering a single proton placed on the methyl symmetry axis at a distance from the methyl group such that the external proton relaxation contributions are reproduced; in the case of a single proton positioned in this manner the auto and cross-correlation spectral densities that result from external proton-methyl proton interactions also become equivalent, with methyl averaging that results from rotation about the symmetry axis subsumed into the effective distance r_{HH_E}. Using r_{HC} = 1.1 Å, r_{HH} = 1.8 Å it follows that j_{CH}/j_{HH}~0.53 so that Eq (S6) can be recast as

$$\hat{R}_{IP}^{G} = R_{\infty}^{\text{int}} \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.86 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.43 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.86 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0.05 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.05 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.86 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.43 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.86 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

$$+R_{\infty}^{ext} \begin{bmatrix} 3 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 9 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 21 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 39 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 9 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 0 & 0 \\ -3 & 0 & 0 & 0 & 0 & 7 & 0 & 0 & 0 & 0 & -4 & 0 & 0 & 0 & 0 & 0 \\ 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 11 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 \\ 0 & 0 & -3 & 0 & 0 & 0 & 0 & 21 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 21 & 0 & 0 & 0 & 0 & -3 & 0 & 0 \\ 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 11 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 \\ 0 & 0 & 0 & 0 & 0 & -4 & 0 & 0 & 0 & 0 & 7 & 0 & 0 & 0 & 0 & -3 \\ 0 & 0 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 9 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 39 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 21 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -2\sqrt{3} & 0 & 0 & 0 & 0 & 9 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 3 \end{bmatrix} \qquad \text{(S8)}$$

where R_{∞}^{int} is the IP DQ relaxation rate, and $21R_{\infty}^{ext}$ is the contribution to the relaxation of DQ elements from external ^{1}H spins. Relaxation rates for the AP terms, corresponding to (elements 17 to 32 of $\vec{v}$) can be obtained from the expressions for the IP rates as follows

$$\hat{R}_{AP}^{G} = \hat{R}_{IP}^{G} + \Delta R_{IP-AP}^{TQ} \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{1}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{-1}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{9} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \frac{-1}{9} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{-1}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{-1}{3} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{-1}{9} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{9} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{3} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{-1}{3} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{3} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \qquad \text{(S9)}$$

where $\Delta R_{IP-AP}^{TQ} = R_{2\infty,IP}^{TQ} - R_{2\infty,AP}^{TQ}$ is the difference between IP and AP methyl ^{1}H TQ coherences that can be measured as described previously (Yuwen, Vallurupalli, and Kay, 2016) or simply treated as a fitting parameter. Finally, the relaxation for all elements of *G* is calculated according to

$$\hat{R}^G = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \otimes \hat{R}_{IP}^G + \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} \otimes \hat{R}_{AP}^G + \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix} \otimes \hat{C}_{IP-AP}^G \tag{S10}$$

where the matrix $\hat{C}_{IP-AP}^G$ contains the coupling terms between the IP and AP density matrix elements that arise due to cross-correlated relaxation between intra-methyl ^{1}H-^{13}C and ^{1}H-^{1}H dipolar interactions,

$$\hat{C}_{IP-AP}^G = j_{HH,HC} \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 3 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 6 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -6 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -3 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix} \tag{S11}$$

In Eq (S11) $j_{HH,HC} = \left(\frac{\mu_o}{4\pi}\right)^2 \frac{1}{15} \frac{\gamma_H^3 \gamma_C \hbar^2}{r_{HC}^3 r_{HH}^3} \tau_C$, derived under the assumption of rapid rotation about the methyl 3-fold symmetry axis. Using standard methyl group geometry, as described above, $j_{HH,HC} \approx 1.37 j_{CH}$ and the coupling matrix can be written in terms of R_∞^{int} so that extra terms need not be introduced. Notably, for reasons described below, the $\hat{C}_{IP-AP}^G$ coupling matrix can be safely ignored. Assuming that $\hat{R}^G = \hat{R}^E$ the full relaxation matrix is given by

$$\hat{R} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \otimes \hat{R}^G \tag{S12}$$

Finally (2-site) chemical exchange is taken into account by including matrix $\hat{K}$

$$\hat{K} = \begin{bmatrix} k_{GE} & -k_{EG} \\ -k_{GE} & k_{EG} \end{bmatrix} \otimes \hat{E}_{32} \tag{S13}$$

where $\hat{E}_{32}$ is a 32×32 identity matrix (McConnell, 1958). In general, we fit DQ and TQ CPMG data together, to extract global exchange parameters k_{ex} and p_E and site-specific parameters $\Delta\varpi$ and relaxation rates R_∞^{int} and R_∞^{ext} from which the elements of $\hat{R}$ are estimated. In fits involving the complete basis set the relaxation parameters R_∞^{int} and R_∞^{ext} are extracted instead of $R_{2\infty,IP}^{DQ}$ and $R_{2\infty,IP}^{TQ}$ (Eq (2)) so that extra fitting parameters are not required.

Dispersion profiles were fit using ChemEx (https://github.com/gbouvignies/chemex) using a new module that is available from the authors upon request. As described above the simpler formalism where only DQ elements are considered (Eq (2)) is sufficient for analysis of experimental data. However, the complete basis permits an evaluation of the effects of relaxation during the CPMG pulses. Figure S1 shows the results of a simulation of DQ CPMG profiles using either regular or composite refocusing pulses in the absence of chemical exchange.

As described above cross-correlated relaxation between intra-methyl ^{1}H-^{13}C and ^{1}H-^{1}H dipolar interactions leads to a coupling between IP and AP elements (Eq (S10) and (S11)). This can be seen most simply from

$$\begin{aligned}
\frac{d}{dt}M_{12,IP} &= -3j_{HH,HC}M_{12,AP} \\
\frac{d}{dt}M_{13,IP} &= -6j_{HH,HC}M_{13,AP} \\
\frac{d}{dt}M_{24,IP} &= 6j_{HH,HC}M_{24,AP} \\
\frac{d}{dt}M_{34,IP} &= 3j_{HH,HC}M_{34,AP}
\end{aligned} \tag{S14}$$

with equivalent additional expressions obtained by replacing ij in $M_{ij,IP}$ above by ji or by interchanging AP and IP in Eq (S14) as shown in equations S10 & S11. Simulations show that when these additional elements are included in $\hat{R}$ there is essentially no change to the evolution of the density matrix and they can therefore be neglected. Insight into why this is the case is provided in what follows where, for simplicity, we neglect chemical exchange and consider only the evolution of double quantum density elements during the CPMG pulse train. Our starting point, therefore, is a basis comprised of in-phase and anti-phase DQ transitions,

$$\vec{v}^{DQ} = [M_{13,IP}\ M_{24,IP}\ M_{31,IP}\ M_{42,IP}\ M_{13,AP}\ M_{24,AP}\ M_{31,AP}\ M_{42,AP}]^T \tag{S15}$$

where the elements in $\vec{v}^{DQ}$ relax via the relaxation matrix

$$\hat{R}^{DQ} = \begin{bmatrix}
R_{2\infty,IP}^{13} & 0 & 0 & 0 & \eta & 0 & 0 & 0 \\
0 & R_{2\infty,IP}^{24} & 0 & 0 & 0 & -\eta & 0 & 0 \\
0 & 0 & R_{2\infty,IP}^{31} & 0 & 0 & 0 & \eta & 0 \\
0 & 0 & 0 & R_{2\infty,IP}^{42} & 0 & 0 & 0 & -\eta \\
\eta & 0 & 0 & 0 & R_{2\infty,AP}^{13} & 0 & 0 & 0 \\
0 & -\eta & 0 & 0 & 0 & R_{2\infty,AP}^{24} & 0 & 0 \\
0 & 0 & \eta & 0 & 0 & 0 & R_{2\infty,AP}^{31} & 0 \\
0 & 0 & 0 & -\eta & 0 & 0 & 0 & R_{2\infty,AP}^{42}
\end{bmatrix} \tag{S16}$$

that includes cross-relaxation between IP and AP DQ elements, for simplicity in what follows denoted by η. Only intra-methyl relaxation is considered in Eq (S14). The effect of each refocusing ^{1}H π pulse during the CPMG train is to interconvert $M_{13,IP}$ & $M_{42,IP}$, $M_{31,IP}$ & $M_{24,IP}$, $M_{13,AP}$ & $M_{42,AP}$ and $M_{31,AP}$ & $M_{24,AP}$. Thus, the evolution of $\vec{v}^{DQ}$ from each pulse is given by $\hat{U}\vec{v}^{DQ}$ where

$$\hat{U} = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}. \tag{S17}$$

Relaxation of the DQ elements during a $\tau_{CP} - \pi - \tau_{CP}$ element, starting from $\vec{v}^{DQ}(0)$, is given by $\vec{v}^{DQ}(2\tau_{CP}) = e^{\hat{R}^{DQ}\tau_{CP}}\hat{U}e^{\hat{R}^{DQ}\tau_{CP}}\vec{v}^{DQ}(0) = \hat{U}e^{\hat{U}^{-1}\hat{R}^{DQ}\hat{U}\tau_{CP}}e^{\hat{R}^{DQ}\tau_{CP}}\,\vec{v}^{DQ}(0)$ with

$$\hat{U}^{-1}\hat{R}^{DQ}\hat{U} = \begin{bmatrix} R^{42}_{2\infty,IP} & 0 & 0 & 0 & -\eta & 0 & 0 & 0 \\ 0 & R^{31}_{2\infty,IP} & 0 & 0 & 0 & \eta & 0 & 0 \\ 0 & 0 & R^{24}_{2\infty,IP} & 0 & 0 & 0 & -\eta & 0 \\ 0 & 0 & 0 & R^{13}_{2\infty,IP} & 0 & 0 & 0 & \eta \\ -\eta & 0 & 0 & 0 & R^{42}_{2\infty,AP} & 0 & 0 & 0 \\ 0 & \eta & 0 & 0 & 0 & R^{31}_{2\infty,AP} & 0 & 0 \\ 0 & 0 & -\eta & 0 & 0 & 0 & R^{24}_{2\infty,AP} & 0 \\ 0 & 0 & 0 & \eta & 0 & 0 & 0 & R^{13}_{2\infty,AP} \end{bmatrix} \tag{S18}$$

Assuming that $e^{\hat{U}^{-1}\hat{R}^{DQ}\hat{U}\tau_{CP}}e^{\hat{R}^{DQ}\tau_{CP}} \approx e^{\hat{U}^{-1}\hat{R}^{DQ}\hat{U}\tau_{CP}+\hat{R}^{DQ}\tau_{CP}}$ it follows that

$$\left(\hat{U}^{-1}\hat{R}^{DQ}\hat{U} + \hat{R}^{DQ}\right)\tau_{CP} = 2\begin{bmatrix} R^{DQ}_{2\infty,IP} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & R^{DQ}_{2\infty,IP} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & R^{DQ}_{2\infty,IP} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & R^{DQ}_{2\infty,IP} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & R^{DQ}_{2\infty,AP} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & R^{DQ}_{2\infty,AP} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & R^{DQ}_{2\infty,AP} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & R^{DQ}_{2\infty,AP} \end{bmatrix}\tau_{CP} \tag{S19}$$

where we have made use of the fact that $R^{13}_{2\infty,IP} = R^{31}_{2\infty,IP}$, $R^{24}_{2\infty,IP} = R^{42}_{2\infty,IP}$, $R^{13}_{2\infty,AP} = R^{31}_{2\infty,AP}$ and $R^{24}_{2\infty,AP} = R^{42}_{2\infty,AP}$ and defined $R^{DQ}_{2\infty,IP} = 0.5\left(R^{13}_{2\infty,IP} + R^{24}_{2\infty,IP}\right)$ and $R^{DQ}_{2\infty,AP} = 0.5\left(R^{13}_{2\infty,AP} + R^{24}_{2\infty,AP}\right)$. Finally, it is worth noting that the difference in relaxation rates between corresponding IP and AP density elements is small, typically less than 0.5 s^{-1}, so that their interconversion via relaxation can have little effect.

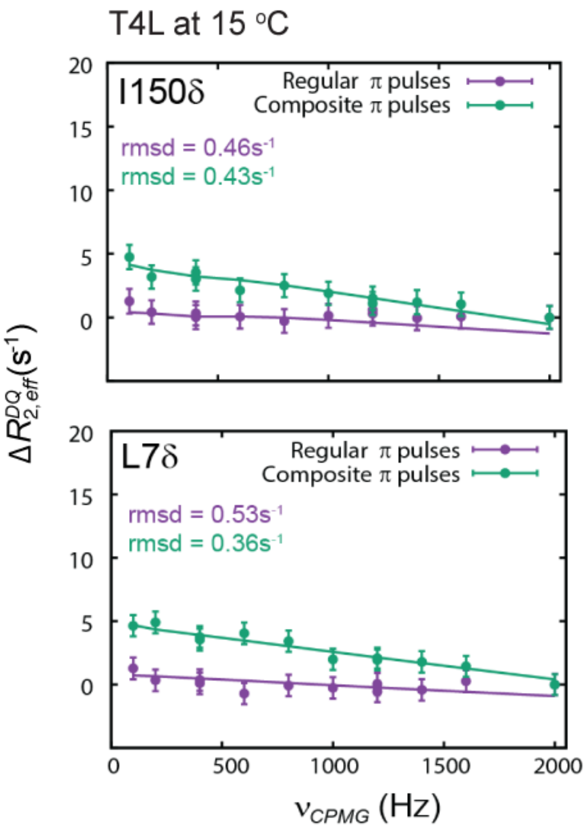

Figure S1 Apparent methyl ^{1}H DQ CPMG "dispersions" can be explained using the Bloch-McConnell equations for a spin 3/2 system. Solid lines were calculated by propagating the Bloch-McConnell equations using a basis set that consists of all transitions in the spin 3/2 manifold with $\Delta\varpi$ = 0 ppm, k_{ex} = 0 s^{-1} and p_E = 0. R_{∞}^{ext} rates were estimated by quantifying the relaxation decay of the 4-quantum element $C_+H_+^1H_+^2H_+^3$ that is immune to relaxation from internal methyl dipolar interactions in the limit that only *j*(0) terms are considered, while R_{∞}^{int} values have been calculated from measured $R_{2\infty,IP}^{TQ}$ rates using the relation $R_{2\infty,IP}^{TQ}$=0.43$R_{\infty}^{int} + 39R_{\infty}^{ext}$, Eq (S8). The calculated profiles, which were generated without any fitting parameters, establish that the origin of the 'dispersions' is differential relaxation of the density elements that are excited during the refocusing pulses and that the effect is larger for the composite pulses than for regular π pulses. Experimental data points are denoted by circles.

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Code to run the DQ CPMG experiment on a Bruker Spectrometer.

```
/* 13CH3_1H_DQ_CPMG_pv_700_cpz, modified from TY's 13CH3_TQ experiment

    This pulse sequence will allow one to perform the following experiment:

    2D 1H/13C to measure exchange via 13CH3 DQ (1H) CPMG

    Assumes that sample is specifically 13CH3 labeled

        1H: O1 on water (~4.7ppm) at end, most of the time at ~0.8 ppm (methyl 1H resonances)
            pwh = p1 1H pw90 @ power level pl1 highest power
            pwh_cp = p15 1H pw90 @ power level pl15 for CPMG pulses

       13C: O2 centre at 20 ppm
            pwc = p2 13C pw90 @ power level pl2 highest power
            power level pl21 is used for 13C decoupling.

        2H: O4 centre at ~0.8 ppm (methyl 1H resonances)
            pwd = p4 2H pw90 @ power level pl4 high power
            power level pl41 is used for 2H waltz-16 decoupling.
            Note: 2H decoupling only necessary for measuring nuCPMG < 100 Hz

       Recommend: use -Dcomp_flg to apply composite 180o CPMG pulses

       TQCPMG written by T. Yuwen on Nov 21, 2016
       DQCPMG written by PV on August 31st 2017
       Added Shared CT evolution on 21/May/2018
*/

prosol relations=<triple>

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>

/***********************/
/*   Define phases     */
/***********************/
#define zero ph=0.0
#define one ph=90.0
#define two ph=180.0
#define three ph=270.0

/***********************/
/*   Define pulses     */
/***********************/
define pulse dly_pg1     /* Messerle purge pulse */
  "dly_pg1=2m"
define pulse dly_pg2     /* Messerle purge pulse */
  "dly_pg2=3.4m"
define pulse pwh
  "pwh=p1"               /* 1H hard pulse at power level p1 (tpwr) */
define pulse pwc
  "pwc=p2"               /* 13C pulse at power level pl2 (dhpwr) */

#ifdef Ddec
define pulse pwd
  "pwd=p4"               /* 2H pulse at power pl4 */
#endif

define pulse pwh_cp      /* 1H CPMG pulse power level */
  "pwh_cp=p15"

#ifdef water_flg
  define pulse pw_sl1
    "pw_sl1=p14"         /* Eburp1 pulse, ~7000 us */
#endif

/************************/
/*   Define delays      */
/************************/
"in0=inf1/2"
"d11=30m"
```

```
"d19=abs(1.0/cnst1)/4"   /* Delay time for 3-9-19 WATERGATE */

/************************/
/*   Define f1180       */
/************************/
#ifdef f1180
  "d0=larger((in0)/2, 0.2u)"
#else
  "d0=0.0"
#endif

define delay hscuba      /* length of 1/2 scuba delay */
  "hscuba=25m"
define delay taua
  "taua=d3"              /* d3 ~ 1.8-2ms ~ 1.0s/(4*125.3)"  ~ 1 / 4J(CH) */
define delay taub
  "taub=d4"              /* d4 = 1/4JCH exactly */

#ifdef cal_flg
define delay tauc
  "tauc=1.00m"
#endif

define delay time_T2
  "time_T2=d6"           /* CPMG duration <= 40 ms  */

define delay time_eq
"time_eq=d14"

/***********************************/
/* Define parameters related to CPMG */
/***********************************/
define delay tauCPMG
define delay tauCPMG1
define delay tauCPMG2

define list<loopcounter> ncyc_cp=<$VCLIST>

"cnst31=1/time_T2"       /* minimum nuCPMG for given time_T2 */

/***************************************************/
/* Assign cnsts to check validity of parameter ranges */
/***************************************************/
#ifdef fsat
  "cnst10=plw10"         /* tsatpwr pl10 - set max at 0.00005W */
#endif

#ifdef mess_flg
  "cnst11=plw11"         /* tpwrmess pl11 - set max at 1.0W */
#endif

#ifdef water_flg
  "cnst14=spw14"         /* power level for eburp1 pulse preeceding start of sequence */
#endif

"cnst15=plw15"           /* tpwrcp - power level for 1H CPMG pulses */
"cnst21=plw21"           /* dpwr pl21 - set max at 2.0W */
"cnst2=plw2"             /* dpwr pl2 - set max at 102.W */

#ifdef Ddec
  "cnst4=plw4"           /* dpwr3 pl4 - set max at 10.5W */
  "cnst41=plw41"         /* dpwr3D pl41 - set max at 1.5W */
#endif

/***************************/
/* Define 3-9-19 WATERGATE */
/***************************/
#define Watergate (center (pwh*0.231 ph26 d19*2 pwh*0.692 ph26 d19*2 pwh*1.462 ph26 d19*2 pwh*1.462 ph28 d19*2
pwh*0.692 ph28 d19*2 pwh*0.231 ph28):f1 (pwc*2 ph26):f2)

/****************************/
/* Initialize loop counters */
/****************************/
"l1=0"
"l2=0"
"l3=0"

;aqseq 321

1 ze
```

```
/****************************************************************/
/* Check validity of parameters and assign values to some of them */
/****************************************************************/
#ifdef Ddec
  if "d1 < 2.0s"
  {
    2u
    print "error: d1 is too short when applying 2H decoupling"
    goto HaltAcqu
  }
#endif

#ifdef fsat
  if "cnst10 > 0.00005"
  {
    2u
    print "error: tpwrmess pl10 too large"
    goto HaltAcqu
  }
#endif

#ifdef mess_flg
  if "cnst11 > 1.0"
  {
    2u
    print "error: tpwrmess pl11 too large"
    goto HaltAcqu
  }
#endif

  if "dly_pg1 > 10m"
  {
    2u
    print "error:dly_pg too long !!"
    goto HaltAcqu
  }

#ifdef water_flg
  if "cnst14 > 0.006"
  {
    2u
    print "error: power level for eburp1 pulse is too large"
    goto HaltAcqu
  }
#endif

  if "cnst15 > 7.5"
  {
    2u
    print "error: 1H CPMG power pl15 too large < 7.5 W"
    goto HaltAcqu
  }

  if "time_T2 > 40.1m"
  {
    2u
    print "error: time_T2 too long < 41ms"
    goto HaltAcqu
  }



  if "cnst21 > 0.9"
  {
    2u
    print "error: dpwr pl21 too large"
    goto HaltAcqu
  }


  if "cnst2 > 102.1"
  {
    2u
    print "error: dpwr pl2 too large, Dont fry the probe!"
    goto HaltAcqu
  }



  if "aq > 0.065s"
```

```
  {
    2u
    print "error: aq is too long; < 65 m"
    goto HaltAcqu
  }

#ifdef Ddec
  if "cnst4 > 10.5"
  {
    2u
    print "error: dpwr3 pl4 too large"
    goto HaltAcqu
  }

  if "cnst41 > 0.9"
  {
    2u
    print "error: dpwr3D pl41 too large"
    goto HaltAcqu
  }

  if "pwd > 160u"
  {
    2u
    print "error: pwd is too large < 160u"
    goto HaltAcqu
  }

  if "pwd < 100u"
  {
    2u
    print "error: pwd is too small > 100u"
    goto HaltAcqu
  }

; d11 LOCKDEC_ON /* Not required for AvanceIII-HD */
  50u LOCKH_ON
  d11 H2_PULSE
  2u pl41:f4
#endif

2 d11 do:f2
/****************************************/
/* Continue to check run time variables */
/****************************************/
  "l2 = (trunc(ncyc_cp[l1] + 0.3))"

  if "l2 > 80"
  {
    2u
    print "error: ncyc_cp must be < 81"
    goto HaltAcqu
  }

  if "l2 != 0"
  {
    "tauCPMG = time_T2*0.25/l2"
    if "tauCPMG < 124.0u"
    {
      2u
      print "error: tauCPMG < 124u too short for CPMG"
      goto HaltAcqu
    }
  }
  else
  {
    "tauCPMG = time_T2*0.25"
  }

  "tauCPMG1 = tauCPMG - pwh_cp*2.0"
#ifdef comp_flg
  "tauCPMG2 = tauCPMG - pwh_cp*2.0"
#else
  "tauCPMG2 = tauCPMG - pwh_cp"
#endif

  20u fq=0:f1 fq=0:f2
  20u pl1:f1 pl2:f2

#ifdef Ddec
```

```
  d11 H2_LOCK
  6m LOCKH_OFF
#endif /*Ddec*/

/******************/
/* Messerle purge */
/******************/
#ifdef mess_flg
  20u pl11:f1
  (dly_pg1 ph26):f1
  20u
  (dly_pg2 ph27):f1
  20u pl10:f1
#endif

/*****************/
/* Presaturation */
/*****************/
#ifdef fsat
  4u pl10:f1
  d1 cw:f1 ph26
  4u do:f1
  2u pl1:f1
#ifdef fscuba
  hscuba
  (pwh ph26):f1
  (pwh*2 ph27):f1
  (pwh ph26):f1
  hscuba
#endif /*fscuba*/
#else
  2u pl1:f1
  d1
#endif /*fsat*/

#ifdef Ddec
  50u LOCKH_ON
  15u H2_PULSE
#endif

/**************************/
/* Water selective Eburp1 */
/**************************/
#ifdef water_flg
  2u
  (pw_sl1:sp14 zero):f1
  2u
#endif

/******************************************/
/* Destroy 13C equlibrium magnetization */
/******************************************/
  (pwc zero):f2

#ifdef Ddec
  20u UNBLKGRAMP
#else
  20u UNBLKGRAD
#endif

  2u
  p50:gp0
  d16

/***********************/
/* Create DQ coherence */
/***********************/
  20u fq=cnst1:f1 pl1:f1
  2u rpp11 rpp12 rpp13 rpp14 rpp15

  (pwh ph1):f1 ; Start INEPT

  2u
  p51:gp1
  d16

  "DELTA = taua - 2u - p51 - d16"
  DELTA

  (center (pwh*2 ph1):f1 (pwc*2 zero):f2)
```

```
  2u
  p51:gp1
  d16

  "DELTA = taua - 2u - p51 - d16"
  DELTA

  (pwh ph6):f1            ; End of INEPT


  2u
  p52:gp2
  d16

  (pwc zero):f2                ; Start refocused INEPT


  2u
  p53:gp3
  d16

  "DELTA = taub - 2u - p53 - d16"
  DELTA

  (center (pwh*2 ph1):f1 (pwc*2 ph26):f2)

  2u
  p53:gp3
  d16

  "DELTA = taub - 2u - p53 - d16"
  DELTA

  (pwc one):f2        ; End of refocused INEPT

        20u pl15:f1
  2u
  p54:gp4
  d16

        "DELTA = time_eq"
        DELTA

#ifdef Ddec
  2u pl4:f4
  (pwd ph27):f4
  2u pl41:f4
  (2u cpds4 ph26):f4
#endif


  (pwh_cp ph1):f1

/*********************************************************/
/* Option to measure differential relaxation for AP/IP DQ */
/*********************************************************/
#ifdef cal_flg
  if "l3%2 == 0" {
    "DELTA = tauc - pwh_cp*2.0"
    DELTA
    (pwh_cp ph29 pwh_cp*2 ph26 pwh_cp ph29):f1
    DELTA
  }
  else {
    tauc
    tauc
  }
#endif

/*********************************/
/* The first half of CPMG period */
/*********************************/
  if "l2 == 1" {
    tauCPMG1
    (pwh_cp ph29 pwh_cp*2 ph26 pwh_cp ph29):f1
    tauCPMG1
  }

  if "l2 > 1" {
```

```
3   tauCPMG2
#ifdef comp_flg
    (pwh_cp ph14 pwh_cp*2 ph11 pwh_cp ph14):f1
#else
    (pwh_cp*2 ph11):f1
#endif
    tauCPMG2 ipp11 ipp12 ipp13 ipp14 ipp15
    lo to 3 times l2
  }

/****************************/
/* The central 180o 1H pulse */
/****************************/
  (pwh_cp*2 ph2):f1


/*********************************/
/* The second half of CPMG period */
/*********************************/
  if "l2 == 1" {
    tauCPMG1
    (pwh_cp ph27 pwh_cp*2 ph26 pwh_cp ph27):f1
    tauCPMG1
  }

  if "l2 > 1" {
4   tauCPMG2 dpp11 dpp12 dpp13 dpp14 dpp15
#ifdef comp_flg
    (pwh_cp ph13 pwh_cp*2 ph12 pwh_cp ph13):f1
#else
    (pwh_cp*2 ph12):f1
#endif
    tauCPMG2
    lo to 4 times l2
  }

/*******************************************************/
/* Option to measure differential relaxation for AP/IP DQ */
/*******************************************************/
#ifdef cal_flg
  if "l3%2 == 0" {
    "DELTA = tauc - pwh_cp*2.0"
    DELTA
    (pwh_cp ph27 pwh_cp*2 ph26 pwh_cp ph27):f1
    DELTA
  }
  else {
    tauc
    tauc
  }
#endif

  (pwh_cp ph7):f1    /* Change 45 if you changing phase cycle */

#ifdef Ddec
  2u do:f4
  2u pl4:f4
  (pwd ph29):f4
#endif

  2u
  p55:gp5
  d16

       "DELTA = time_eq"
       DELTA

/* ----- End of CPMG ---*/

  20u pl1:f1

/* ------ Shared CT ----*/
  (pwc ph3):f2                ; t1 evolution

  if "d0 <= taub" {
               "DELTA = taub + d0 + 1u"
       DELTA
       (center (pwh*2 ph26):f1 (pwc*2 ph26):f2)
               "DELTA = taub - d0 + 1u"
       DELTA
```

```
  }
  else {
             "DELTA = taub + d0 -pwh"
             DELTA
             (pwh*2 ph26):f1
             "DELTA = d0 - taub"
             DELTA
             1u
             (pwc*2 ph26):f2
             "DELTA = 1u+pwh"
             DELTA
  }
  (pwc ph4):f2

  2u
  p58:gp8
  d16

/*****************************************************************/
/* C->H back transfer, use wtg_flg for better water suppression */
/*****************************************************************/
  20u fq=0:f1 pl1:f1

  (pwh one):f1

#ifdef wtg_flg
  2u
  p60:gp10
  d16

  "DELTA = taua - 2u - p60 - d16 - d19*3.367 - pwh*2.0/PI"
  DELTA

  Watergate

  2u
  p60:gp10
  d16

  "DELTA = taua - 2u - p60 - d16 - 4u - de - pwc*2 - 4u - d19*3.367"
  DELTA
#else
  2u
  p59:gp9
  d16

  "DELTA = taua - 2u - p59 - d16"
  DELTA

  (center (pwh*2 zero):f1 (pwc*2 zero):f2)

  2u
  p59:gp9
  d16

  "DELTA = taua - 2u - p59 - d16 - 4u - de - pwc*2 - 4u"
;  "DELTA = taua - 2u - p55 - d16 - 4u - pwc*2 - 4u"


   DELTA
#endif

#ifdef Ddec
  4u BLKGRAMP
#else
  4u BLKGRAD
#endif

  (pwc ph26):f2
  (pwc ph5):f2

  4u pl21:f2             /* lower power for 13C decoupling */

#ifndef wtg_flg
  (pwh one):f1
#endif

/*******************************/
/* Signal detection and looping */
/*******************************/
```

```
  go=2 ph31 cpds2:f2
  d11 do:f2 mc #0 to 2

#ifdef cal_flg
  F3QF(calclc(l3,1))
#else
  F3QF(calclc(l3,0))
#endif
  F2QF(calclc(l1,1))
  F1PH(calph(ph4,-90), caldel(d0,+in0) & calph(ph31,+180))

#ifdef Ddec
  d11 H2_LOCK
  d11 LOCKH_OFF
; d11 LOCKDEC_OFF        /* use statement for earlier hardware */
#endif

HaltAcqu, 1m
exit

ph0=1
ph1=(8) 7 1           ; phi1
ph6=(8) 1 3           ; phi1+90
ph7=(8) 7             ; -45 pulse at the end of cpmg
ph2=1 1 3 3           ; central 180 in the cpmg
ph3=0 0 0 0 2 2 2 2   ; pulse at the start of t1
ph4=1
ph5=0 2               ; c13 pulse before detection
ph11={0 1 0 1 1 0 1 0}^2  ;xy16
ph12={0 3 0 3 3 0 3 0}^2  ;mxy16
ph13=ph12+ph0
ph14=ph11-ph0
ph15={{{0 2}^2}^2}^2
ph26=0
ph27=1
ph28=2
ph29=3
ph31=0 2 0 2 2 0 2 0  ; receiver

;pl1 : tpwr - power level for pwh
;pl2 : dhpwr - power level for 13C pulse pwc (p2)
;pl4  : power level for 2H high power pulses
;pl10 : tsatpwr - power level for presat
;pl11 : tpwrmess - power level for Messerle purge
;pl15 : power level for 1H CPMG pulses pwh_cp
;pl21 : dpwr - power level for  13C decoupling cpd2
;pl41 : power level for 2H waltz decoupling
;spw14 : power level for eburp1 pulse
;spnam14: eburp1 pulse on water
;p1 : pwh
;p2 : pwc
;p4 : 2H high power pulse
;p14 : eburp1 pulse width, typically 7000u
;p15 : 1H pw for CPMG pulses
;p50 : gradient pulse 50                                [1000 usec]
;p51 : gradient pulse 51                                [300 usec]
;p52 : gradient pulse 52                                [500 usec]
;p53 : gradient pulse 53                                [300 usec]
;p54 : gradient pulse 54                                [500 usec]
;p55 : gradient pulse 55                                [500 usec]
;p56 : gradient pulse 56                                [300 usec]
;p57 : gradient pulse 57                                [500 usec]
;p58 : gradient pulse 58                                [500 usec]
;p59 : gradient pulse 59                                [300 usec]
;p60 : gradient pulse 60                            [400 usec]
;pcpd2 : 13C pulse width for 13C decoupling
;pcpd4 : 2H pulse width for 2H decoupling
;d1 : Repetition delay D1
;d3 : taua ~1/(4*JCH)  ~1.8-2ms
;d4 : taub - set to 1/4JHC = 2.0 ms
;d6 : time_T2 CPMG duration <= 40ms
;d11 : delay for disk i/o, 30ms
;d16 : gradient recovery delay, 200us
;d19: delay for binomial water suppression, = 1/(4*|cnst1|)
;d14: time_eq 2 - 5ms
;cpd2 : 13C decoupling during t2 according to program defined by cpdprg2
;cpd4 : 2H decoupling during t1
;cpdprg2 : 13C decoupling during t2
;cpdprg4 : 2H decoupling during t1
;cnst1 : offset of methyls from water (0.8 ppm - 4.7 ppm, in Hz)
```

```
;cnst2 : power for 13C hard pulse
;cnst4 : power level for 2H high power pulses
;cnst14 : power level in w for 1H selective pulse
;cnst15 : power in w for 1H CPMG pulses
;cnst21 : power in w for 13C dec
;cnst31 : minimum nuCPMG for given time_T2
;cnst41 : power level for 2H decoupling
;l1 : counter for the ncyc_cp values for cpmg
;l2 : actual value of ncyc_cp
;l3 : flag to switch between IP and AP for -Dcal_flg
;inf1 : 1/SW(X) = 2*DW(X)
;in0 : 1/(2*SW(x))=DW(X)
;nd0 : 2
;ns : 4*n
;FnMODE : States-TPPI, TPPI, States

;for z-only gradients:
;gpz0: 20%
;gpz1: 25%
;gpz2: 27%
;gpz3: 30%
;gpz4: 40%
;gpz5: 50%
;gpz6: -27%
;gpz7: 43%
;gpz8: 47%
;gpz9: 33%
;gpz10: -50%

;use gradient files:
;gpnam0: SMSQ10.32
;gpnam1: SMSQ10.32
;gpnam2: SMSQ10.32
;gpnam3: SMSQ10.32
;gpnam4: SMSQ10.32
;gpnam5: SMSQ10.32
;gpnam6: SMSQ10.32
;gpnam7: SMSQ10.32
;gpnam9: SMSQ10.32
;gpnam9: SMSQ10.32
;gpnam10: SMSQ10.32

;zgoptns: Dfsat Dmess_flg Dfscuba Dwater_flg Dwtg_flg Df1180 DDdec Dcomp_flg Dcal_flg
```