Accessing Long Lived ^1H States via ^2H Couplings

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Abstract

In this paper we demonstrate long-lived states involving a pair of chemically equivalent protons, with lifetimes ~ 30 times T_1 up to a total lifetime of ~ 117 s at high field (8.45 T). This is demonstrated on *trans*-ethylene- d_2 in solution, where magnetic inequivalence gives access to the long-lived states. It is shown that the remaining J -coupling between the two quadrupolar deuterium spins, J_{QQ} , splits the conditions for optimally generating proton singlet states. Detailed simulations of the spin evolution are performed, shedding light on the coherent evolution during singlet-triplet conversion as well as on the incoherent evolution that causes relaxation. Subsequently, the simulations are compared with experimental results validating the theoretical insights. Possible applications include storage of hyperpolarization in the proton long-lived state. Of particular interest may be utilization of parahydrogen induced polarization to directly induce the examined long-lived states.

Keywords

Solution NMR; Long lived states; Ethylene; Deuterium

1. Introduction

Disconnected eigenstates, such as singlet states, with very long lifetimes as compared to usual T_1 times have previously been shown to exist on coupled spin-1/2 pairs [1–11]. Some stellar examples include a naphthalene derivative with ^{13}C spins supporting a lifetime in excess of 1 hour (~ 50 times T_1) [8]. In that specific case, a chemical shift difference between the coupled spins is used to access the singlet state population on the spin-pair. Examples also exist in which the singlet state population is stored in chemically equivalent spins (typically ^{13}C and ^{15}N), where couplings to auxiliary spins are used (typically ^1H) to break the magnetic equivalence [5,7]. For example, diphenyl- $^{13}\text{C}_2$ -acetylene contains chemically equivalent ^{13}C spins coupled to aromatic ring protons. This system produces a long-lived state lifetime on the $^{13}\text{C}_2$ spin pair of around 5 min (~ 21 times the carbon T_1) [5]. These are advantageous spin systems because the long-lived state is stable at high fields and because it can be loaded and unloaded using only pulses on the auxiliary protons, which makes the long-lived $^{13}\text{C}_2$ spin state accessible in any clinical imager without requiring a dedicated ^{13}C channel.

Recently it was shown, that the ^{13}C singlet state can also be accessed by auxiliary spin-1 nuclei, such as ^2H [12]. The previously demonstrated systems had a particularly simple coupling network, since the deuterium atoms were highly separated so that deuterium-deuterium coupling could be neglected. Here we show for the first time that long-lived states on ^1H can be accessed

by coupling to ^2H ; specifically, we study *trans*-deuterated ethylene in solution, and show that the singlet lifetime of the protons is ~ 30 times T_1 and can reach up to 117 s. Our finding is particularly interesting for hyperpolarization storage. In particular, one common hyperpolarization method (para-hydrogen induced polarization or PHIP) intrinsically produces proton singlets in closely related geometries when the protons are added to equivalent carbons such as $\text{DC}\equiv\text{CD}$ or $\text{CDR}=\text{CRD}$.

Previous work has shown that ethylenic hydrogens can support very long lived states. PHIP reaction with acetylene [13] produced 1% hyperpolarized ethylene which was bubbled through perfluoro (para-tolylsulfenyl) chloride, or through a nematic liquid crystal solvent to make the hyperpolarization NMR detectable (via an induced chemical shift or residual dipolar couplings). In this system, despite a sub-second T_1 , the authors were able to observe several lifetime components in the decay to equilibrium (including a nearly 1000 s component at atmospheric pressure). They also observed immediate PHIP signals from *trans*-ethylene- d_2 ($\text{CDH}=\text{CHD}$) (Figure 1), but with short singlet lifetime of around 8 s. In a related study [14] 1% hyperpolarized propane was produced from *para*-hydrogen and propene, and imaged at both high (4.7 T) and low (0.0475 T) field. The short lifetime at high field (0.6 s) was extended by unlocking long-lived spin states at low field, giving a lifetime of 4.7 s. In propane the two added protons are not equivalent, so observation of a significant extension was surprising.

Here we use the concepts of magnetic equivalence and chemical equivalence to understand ethylene and the *trans*-deuterated species in detail. In ethylene, all four protons are magnetically equivalent, so the spectrum has only a single line. The 16 energy levels can be written as a spin-2 state and two spin-0 states, all with A_g symmetry, plus three spin-1 states (symmetries B_{1u} , B_{2u} and B_{3g} respectively) [13]; only the composition of the two spin-0 eigenstates is affected in any way by the numerical value of scalar (or dipolar) couplings. Reference [23] argues that the long-lived component comes from equilibration between B_{1u} and B_{2u} , which is formally a slow relaxation pathway possibly leading to long lifetimes, however, in ethylene, conversion between the long-lived state and the detectable state cannot be controlled by RF pulses, because all spins are magnetically equivalent. Therefore, total spin is a good quantum number and states with different symmetry are never connected by the Hamiltonian.

In contrast, in the *trans*-ethylene- d_2 species (Figure 1) the two remaining protons are chemically equivalent (no chemical shift difference), but their coupling to the near and far deuterium breaks the magnetic equivalence forming an $AA'QQ'$ system (A being the spin-1/2 and Q the spin-1 species). The $AA'QQ'$ geometry supports long-lived disconnected eigenstates, particularly singlets on the A spins, coupled to a variety of different Q states [12]. A condition for a long lived singlet is that the singlet is close to an eigenstate of the spin system. This is fulfilled if the like-spin couplings (AA and QQ) dominate the unlike-spin couplings (AQ), and as illustrated in Figure 1 below, the *trans* deuteration creates a system with a single dominant scalar coupling, the proton-proton coupling J_{HH} .

Figure 1: A) Ethylene with J -couplings shown. J_n and J_f are the near and far J -couplings between protons. B) *Trans*-ethylene- d_2 . J -couplings are shown in Hz. J_{HH} in B is the same as in A; the near and far proton-deuterium scalar couplings are reduced by (γ_D/γ_H) , the deuterium-deuterium coupling is reduced by $(\gamma_D/\gamma_H)^2$.

2. Methods

Trans-ethylene- d_2 gas was delivered into a high pressure NMR tube containing 500 μL per-deuterated hexane as solvent, with an initial tube pressure of ~ 150 psi. We measured the ^{13}C (natural abundance) spectrum of sample and solvent to determine the ethylene: hexane ratio and found a signal ratio of 1:28. Hexane has density 0.65 g/ml and neat concentration is 7.54 M, so the concentration of ethylene was ~ 270 mM ($7.54/28$). Subsequent concentrations (i.e., equilibrium vapor pressure) were obtained by releasing a fraction of the gas. The remaining concentration was inferred by the relative amount of proton intensity of the ethylene peaks compared to the initial high pressure proton measurement. Experiments were performed on a Bruker 8.45 T (360 MHz) spectrometer running Topspin 1.1. The deuterium lock needed to be turned off to avoid scrambling of the spin states in our sample.

The magnetization to singlet to magnetization (MSM) pulse sequence, first introduced by Levitt and coworkers [2,11,15], and shown in Figure 2, was used to transfer population into and out of the singlet state. This sequence consists of a resonant train of π pulses that, for the chemically equivalent but magnetically inequivalent spin system discussed here, allows the difference in out-of-pair J -couplings ($\Delta J = J_{AQ} - J_{AQ'}$) (see Figure 1) to drive the transition between magnetization and singlet. The two critical parameters for this transition are the interpulse delay, τ , and the number of π pulses, n , depicted in Fig. 2. These are determined by the relative strengths of J_{AA} , J_{AQ} , $J_{AQ'}$ and J_{QQ} , as derived in the next section. The numerical values of the J -couplings were first estimated from the spectra and then refined by experimentally varying both τ and n from which we could, in turn, get more accurate estimates of the J -coupling values.

Figure 2: The pulse sequence used for these experiments. A $\pi/2$ pulse creates transverse magnetization, which is then transferred to singlet order by the M2S (magnetization to singlet) block. The singlet is allowed to relax for a time τ_s , after which residual magnetization is destroyed by three $\pi/2$ -gradient filters. The singlet (having no dipole moment) is left unaffected and subsequently read-out by the S2M (singlet to magnetization) block (S2M is a time-reversed copy of the M2S). The entire sequence is referred to as MSM sequence.

Post processing was performed in MATLAB (Release 2015a. The MathWorks, Inc., Natick, Massachusetts, United States). The singlet decay (spectral extrema vs. τ_s) was fit to a bi-exponential decay. This allowed us to separately resolve any residual rapidly decaying triplet contribution from the long-lived state.

Simulations of the spin system were performed using the SPINACH MATLAB package [16–19]. The outputs of a Gaussian09 (Revision D.01 Gaussian, Inc. Wallingford, Connecticut) simulation of *trans*-ethylene- d_2 molecular structure and NMR parameters were fed into SPINACH for NMR sequence simulation. The basis set of the density functional theory calculation used for the Gaussian09 simulation are gauge invariant atomic orbitals (GIAO) [20], hybrid functional (B3LYP) [21] and extension of Gaussian-type basis set (6-31G(df, 3p)) [22]. For full Gaussian09 simulation parameter sets and returned values, please see the appendix. The J -couplings returned by Gaussian09 differed slightly from the couplings determined in experiment; GAUSSIAN output: $J_{HH} = 19.94$ Hz, $J_{HD} = 1.89$ Hz, $J_{HD}' = 0.35$ Hz, $J_{DD} = 0.47$ Hz; while the experimental values are: $J_{HH} = 19.10$ Hz, $J_{HD} = 1.73$ Hz, $J_{HD}' = 0.35$ Hz, $J_{DD} = 0.45$ Hz.

Theory

2.1 The effect of J_{DD}

In our previous studies of the AA'QQ' spin system, for example, deuterated diacetylene, the D-D coupling was insignificant and hence ignored. In *trans*-ethylene- d_2 , the D-D coupling cannot be ignored; it creates multiple resonance conditions (i.e., different τ 's within the π pulse train create singlet order). Let's analyze this in detail.

First consider the combined angular momentum basis for the two A or Q nuclei: $|j_1, m_1, j_2, m_2\rangle = |F, M\rangle$, with F representing the total angular momentum number and M the magnetic quantum number. For the two spin-1/2 protons (AA') the $|F, M\rangle$ states are represented as the singlet-triplet basis ($|0, 0\rangle$, $|1, +1\rangle$, $|1, 0\rangle$, $|1, -1\rangle$, or S , T_{+1} , T_0 , T_{-1} , respectively). For the

two spin-1 deuterons (QQ') the $|F, M\rangle$ states are represented, in analogy, with F ranging from 0 to 2 and M_D (magnetic quantum number of deuterons) ranging from $-F$ to F (The $|F, M\rangle$ states always represent the combined angular momentum basis of two spins). To obtain the combined angular momentum basis for QQ', we first define the three spin states of the individual spin-1 nuclei as P, Z and M (Plus, Zero and Minus). Then, their combined angular momentum basis is given as:

for the $F = 2$ manifold:

$$|2, 2\rangle = PP$$

$$|2, 1\rangle = (PZ + ZP)/\sqrt{2}$$

$$|2, 0\rangle = (PM + 2ZZ + MP)/\sqrt{6}$$

$$|2, -1\rangle = (MZ + ZM)/\sqrt{2}$$

$$|2, -2\rangle = MM$$

for the $F = 1$ manifold:

$$|1, 1\rangle = (PZ - ZP)/\sqrt{2}$$

$$|1, 0\rangle = (PM - MP)/\sqrt{2}$$

$$|1, -1\rangle = (MZ - ZM)/\sqrt{2}$$

for the $F = 0$ manifold:

$$|0, 0\rangle = (PM - ZZ + MP)/\sqrt{3}$$

Combination of these 9 states from two deuterons with the singlet-triplet basis (4 states) of two protons, gives a total of 36 states for the entire spin system.

Now consider the interaction Hamiltonian of this AA'QQ' system, where the heteronuclear J -coupling term may be split into sum and difference terms:

$$\begin{aligned}\hat{H}_{\text{interaction}} &= \hat{H}_{AA} + \hat{H}_{QQ} + \hat{H}_{AQ} + \hat{H}_{AQ'} \\ &= 2\pi J_{AA} \hat{S}_1 \cdot \hat{S}_2 + 2\pi J_{QQ} \hat{I}_1 \cdot \hat{I}_2 + 2\pi J_{AQ} (\hat{I}_{1z} \hat{S}_{1z} + \hat{I}_{2z} \hat{S}_{2z}) + 2\pi J_{AQ'} (\hat{I}_{1z} \hat{S}_{2z} + \hat{I}_{2z} \hat{S}_{1z}) \\ &= 2\pi J_{AA} \hat{S}_1 \cdot \hat{S}_2 + 2\pi J_{QQ} \hat{I}_1 \cdot \hat{I}_2 \\ &\quad + \pi (J_{AQ} + J_{AQ'}) (\hat{I}_{1z} + \hat{I}_{2z}) (\hat{S}_{1z} + \hat{S}_{2z}) + \pi (J_{AQ} - J_{AQ'}) (\hat{I}_{1z} - \hat{I}_{2z}) (\hat{S}_{1z} - \hat{S}_{2z}) \\ &= \hat{H}_{AA} + \hat{H}_{QQ} + \hat{H}_{\Sigma J} + \hat{H}_{\Delta J}\end{aligned}\tag{1}$$

where $(J_{AQ} + J_{AQ'}) = \Sigma J$ and $(J_{AQ} - J_{AQ'}) = \Delta J$.

In the combined angular momentum basis, $\hat{H}_{AA}$, $\hat{H}_{QQ}$ and $\hat{H}_{\Sigma J}$ contribute along the diagonal, while $\hat{H}_{\Delta J}$ is the only term that contributes to the off-diagonal. The eigenvalues for $\hat{H}_{AA}$ are $1/2\pi J_{AA}$ (for T_{+1} , T_0 , T_{-1}) and $-3/2\pi J_{AA}$ (for S), but we shift them by $1/2$ times the identity to provide integer matrix elements. Since the identity commutes with all operators, this has no effect on the evolution of the system. Similarly, the eigenvalues of $\hat{H}_{QQ}$ are $2\pi J_{QQ}$ for the $F=2$ states, $-2\pi J_{QQ}$ for the $F=1$ states and $-4\pi J_{QQ}$ for the single $F=0$ state. Finally, $\hat{H}_{\Sigma J}$ captures the value of M . Since the matrices presented below describe transitions with $\Delta M_I = \Delta M_S = 0$ states, this term is proportional to the identity and is neglected.

These mathematical transformations give us the ability to quantify and understand the conversion of observable magnetization into long lived singlet order in detail. A simplification occurs because, only several individual blocks are connected by the interaction Hamiltonian through the $\hat{H}_{\Delta J}$ term in the chosen basis. In the following we will only concern ourselves with all these individual blocks that connect AA'-triplet states (AA' magnetization) to AA'-singlet states, revealing the interconversion dynamics.

We first focus on the $\hat{H}_{\Delta J}$ term connecting $|F, M\rangle$ with $|F \pm 1, M\rangle$. For $M_D \neq 0$ we find four two-level sub-systems, but only two are unique:

$$\begin{array}{cc} & |2,1\rangle T_0 & |1,1\rangle S \\ |2,1\rangle T_0 & \pi(J_{AA} + 2J_{QQ}) & \pi\Delta J \\ |1,1\rangle S & \pi\Delta J & -\pi(J_{AA} + 2J_{QQ}) \end{array} \quad (2)$$

and

$$\begin{array}{cc} & |2,1\rangle S & |1,1\rangle T_0 \\ |2,1\rangle S & -\pi(J_{AA} - 2J_{QQ}) & \pi\Delta J \\ |1,1\rangle T & \pi\Delta J & \pi(J_{AA} - 2J_{QQ}) \end{array} \quad (3)$$

The other two are obtained by replacing $M=1$ with $M=-1$ which yields identical matrices. (These are the two-level systems connecting $|2, -1\rangle T_0 \leftrightarrow |1, -1\rangle S$ which is identical to Equation (2), and $|2, -1\rangle S \leftrightarrow |1, -1\rangle T_0$, identical to Equation (3)). In these subsystems, the resonance conditions for the MSM sequence to transfer population from the T_0 state to the S state is given by:

$$\tau = \frac{1}{2\sqrt{(J_{AA} + 2J_{QQ})^2 + (\Delta J)^2}}$$

$$n = \pi / \left(2 \arctan \left(\frac{\Delta J}{J_{AA} + 2J_{QQ}} \right) \right) \quad (4)$$

for Equation (2). Similarly:

$$\tau = \frac{1}{2\sqrt{(J_{AA} - 2J_{QQ})^2 + (\Delta J)^2}}$$

$$n = \pi / \left(2 \arctan \left(\frac{\Delta J}{J_{AA} - 2J_{QQ}} \right) \right) \quad (5)$$

gives the MSM resonance conditions for Equation (3). Note that if J_{QQ} were absent, the resonance conditions of Equations (4) and (5) would be degenerate. However, if J_{QQ} is not negligible (as in the present experiments), they are shifted by a factor of $\pm 2J_{QQ}$ (this is also slightly different from the all spin-1/2 AA'XX' case, where this factor is $\pm J_{XX}$ [7]). Finally, the resonance condition for populating the $|1, \pm 1\rangle S$ states, (Equation (4)), depends on the sum of the couplings, resulting in a shorter interpulse-delay, and a slightly increased number of pulses than populating the $|2, \pm 1\rangle S$ states (Equation (5)).

In the case where $M_D = 0$, there exist two 3-level systems. The first:

	$ 2, 0\rangle T_0$	$ 1, 0\rangle S$	$ 0, 0\rangle T_0$
$ 2, 0\rangle T_0$	$\pi(J_{AA} + 2J_{QQ})$	$2\pi\sqrt{1/3}\Delta J$	0
$ 1, 0\rangle S$	$2\pi\sqrt{1/3}\Delta J$	$-\pi(J_{AA} + 2J_{QQ})$	$2\pi\sqrt{2/3}\Delta J$
$ 0, 0\rangle T_0$	0	$2\pi\sqrt{2/3}\Delta J$	$\pi(J_{AA} - 4J_{QQ})$

(6)

and the second (with the S and T_0 labels interchanged):

	$ 2, 0\rangle S$	$ 1, 0\rangle T_0$	$ 0, 0\rangle S$
$ 2, 0\rangle S$	$-\pi(J_{AA} - 2J_{QQ})$	$2\pi\sqrt{1/3}\Delta J$	0
$ 1, 0\rangle T_0$	$2\pi\sqrt{1/3}\Delta J$	$\pi(J_{AA} - 2J_{QQ})$	$2\pi\sqrt{2/3}\Delta J$
$ 0, 0\rangle S$	0	$2\pi\sqrt{2/3}\Delta J$	$-\pi(J_{AA} + 4J_{QQ})$

(7)

The dynamics of these three level systems are considerably more complicated and depend on the exact ratios between J_{AA} , J_{QQ} and ΔJ . However, the naïve resonance conditions obtained by considering the two two-level systems between the first and second states and the second and third states gives a surprisingly close approximation to the correct resonance conditions for a range of J -coupling values. These are:

$$\begin{aligned}\tau_{1,2} &= \frac{1}{2\sqrt{(J_{AA} + 2J_{QQ})^2 + (2\Delta J / \sqrt{3})^2}} \\ n_{1,2} &= \pi / \left(\arctan \left(\frac{2\Delta J / \sqrt{3}}{J_{AA} + 2J_{QQ}} \right) \right) \sqrt{\quad}\end{aligned}\tag{8}$$

connecting the $|2,0\rangle T_0 \leftrightarrow |1,0\rangle S$ states in Equation (6) and

$$\begin{aligned}\tau_{2,3} &= \frac{1}{2\sqrt{(J_{AA} - J_{QQ})^2 + (2\sqrt{2}\Delta J / \sqrt{3})^2}} \\ n_{2,3} &= \pi / \left(2 \arctan \left(\frac{2\sqrt{2}\Delta J / \sqrt{3}}{J_{AA} - J_{QQ}} \right) \right)\end{aligned}\tag{9}$$

connecting the $|0,0\rangle T_0 \leftrightarrow |1,0\rangle S$ states in Equation (6). Similarly:

$$\begin{aligned}\tau_{1,2} &= \frac{1}{2\sqrt{(J_{AA} - 2J_{QQ})^2 + (2\Delta J / \sqrt{3})^2}} \\ n_{1,2} &= \pi / \left(2 \arctan \left(\frac{2\Delta J / \sqrt{3}}{J_{AA} - 2J_{QQ}} \right) \right)\end{aligned}\tag{10}$$

connects $|2,0\rangle S \leftrightarrow |1,0\rangle T_0$ in Equation (7), and

$$\begin{aligned}\tau_{2,3} &= \frac{1}{2\sqrt{(J_{AA} + J_{QQ})^2 + (2\sqrt{2}\Delta J / \sqrt{3})^2}} \\ n_{2,3} &= \pi / \left(\arctan \left(\frac{2\sqrt{2}\Delta J / \sqrt{3}}{J_{AA} + J_{QQ}} \right) \right)\end{aligned}\tag{11}$$

connects $|0,0\rangle S \leftrightarrow |1,0\rangle T_0$ in Equation (7).

All of the resonance conditions derived here will be overlaid on simulation of the states in the following section.

2.2 Simulations of *trans*-ethylene- d_2

Figure 3 shows a simulation of the population in seven of the nine combined deuterium and proton-singlet ($|F, M\rangle S$) states after the M2S sequence as described in Figure 2. The population is shown as a function of the interpulse delay (τ) and number of pulses in the first CPMG train of the M2S sequence (n). Note that pulses with an ($n = \text{even}$ and $n/2 = \text{odd}$) number of π pulses in the M2S block will produce a singlet signal out of phase as with an ($n = \text{even}$ and $n/2 = \text{even}$) combination, and are correspondingly inverted. This results in a striped pattern due to the equilibrium population offsets for the $M \neq 0$ states, but makes the singlet population always positive. The resonances identified in Equations (4), (5) and (8)-(11) are highlighted by circles. As predicted and observed above, the states that involve $F = 1$ require a shorter interpulse delay time and are more efficiently excited with a marginally greater number of pulses than those with $F = 2$. The $M_D = 0$ states populate at intermediate values of interpulse delay time and still lower pulse number.

Figure 3: State-population generated after an M2S sequence as a function of the number of π pulses in the first block (n), and the interpulse delay τ . The predicted resonance conditions from Equation (4) are marked in blue, Equation (5) in red, and Equations (8)-(11) in black. The $M \neq 0$ states have a striped pattern due to the equilibrium population offset of these states. Since the singlet character is generated out of phase for an even/odd combination of n & $n/2$ pulses compared to an even/even combination, the even/odd combinations are reversed in sign to make the singlet character easier to identify. The J -couplings used were those from the GAUSSIAN simulation: $J_{HH} = 19.94$ Hz, $J_{HD} = 1.89$ Hz, $J_{HD}' = 0.35$ Hz, $J_{DD} = 0.47$ Hz.

Finally, we use the SPINACH NMR simulation software package to gain insight into the relaxation behavior. All the Gaussian09 output for this molecule are fed into SPINACH for relaxation simulation and Kuprov's automated relaxation theory module was used [16-19]. A single rotational correlation time was chosen to be 0.1×10^{-12} s. This value was chosen to match simulated and experimental T_1 times. But also, this value is close to a simple estimate by the Stokes-Einstein relation $\tau_c = 4\pi\eta r^3 / 3k_B T$ ($\sim 0.2 \times 10^{-12}$ s, using a radius, r , of 92 pm and the viscosity, η , of hexane as 3×10^{-4} N·s/m²). Quadrupolar couplings were included in the relaxation simulation, while scalar relaxation of the second kind (SRSK) was not included. Figure 4 plots several ways in which the relaxation of the system can be interrogated. First, Figure 4A shows the Redfield relaxation matrix (in the laboratory frame) for the singlet subsystem. Relaxation to the proton triplet states from any of the proton singlet state is negligible. This plot shows that in addition to the states having self-relaxation (diagonal values), there is also considerably cross relaxation between the states. In particular, cross relaxation causes mixing within the $F = 1$ states, no strong connections to other manifolds are found. The $F = 2$ states mix within each other and also with the $F = 0$ state. The smaller space for cross relaxation indicates that we can expect the $F = 1$ states to have a smaller relaxation rate, or longer lifetime. Figure 4B plots exactly this – the lifetime predicted by the relaxation matrix. As expected, the $F = 1$ states have longer lifetimes. Figure 4B, however, excludes an important effect: coherent evolution between the states. This is included in the lifetimes shown in Figure 4C. Here, a specific $|F, M\rangle_S$ state was prepared and then evolved under the full Liouvillian ($\hat{L} = \hat{H} + i\hat{R}$). Coherent evolution allows some mixing between the states and largely equalizes the lifetimes. Finally, M2S does not generate a single $|F, M\rangle_S$ state, but excites a mixture of states (Figure 3). Figure 4D shows the lifetime of these states as prepared under a specific set of MSM parameters (τ and n), evolved under the Liouvillian, and fit to an exponential. The lifetimes at shorter τ , those corresponding to $F = 1$, have slightly longer ($\sim 10\%$) lifetime.

Figure 4: A) The relaxation matrix for the states $|F, M\rangle S_0$ that form the singlet subsystem as predicted by SPINACH [16-19]. Large positive values along the diagonal give the rates of self-relaxation, while cross-relaxation is indicated by the off-diagonal elements. The $F = 2$ and $F = 0$ subsystems mix via cross-relaxation, which gives them a much faster relaxation rates. Relaxation to the triplet states is negligible. B) Lifetime of the singlet states predicted by only the relaxation matrix. C) Upon evolution under the entire Liouvillian ($\hat{H} + \hat{R}$), coherent mixing between the states equalizes the lifetimes. D) Plot of the lifetimes as determined by evolution under the full Liouvillian for each of the points in Figure 3. The mask is generated by only selecting points that have significant singlet population: $1/5^{\text{th}}$ of the maximum singlet population generated. There is an asymmetry to the mask as Equations (8)-(11) map out resonance conditions that shift to a longer number of pulses and shorter interpulse delay as, for example, J_{HH} increases while ΔJ is held constant. Additionally, the lifetime is slightly longer at the shorter interpulse delay, as suggested by the slightly longer lifetimes of the $F = 1$ states.

3. Results

Figure 5 shows the resonance conditions, reproducing the simulated results (A) of the total singlet generated by M2S alone, (from the sum in Figure 3) and compares it to the singlet experimentally measured (B) after the full MSM sequence as both the interpulse delay and the

number of pulses are swept. As predicted by Equations (4) and (5) there are two distinct resonance conditions at $\tau = 12.6$ and 13.8 ms (0.6 ms less in the simulation due to larger values of J_{HH}). The broad feature in the simulation at low pulse numbers results from the three-level systems, however, they are not seen in the experimental data due to their fast relaxation.

Figure 5: A) SPINACH simulation of the total singlet population generated by the M2S sequence as the interpulse delay and number of pulses are swept. B) Experimental signal surviving after the full MSM sequence (singlet signal) as the interpulse delay and number of pulses are swept. The shift in the resonance conditions to lower τ in the simulation results from the larger J -coupling values used in the simulation as compared to the experiment. The simulated (experimental) values were $J_{HH} = 19.94$ (19.10) Hz, $J_{HD} = 1.89$ (1.73) Hz, $J_{HD}' = 0.35$ (0.35) Hz, $J_{DD} = 0.47$ (0.45) Hz.

Singlet lifetimes acquired across these two resonance conditions are shown in Figure 6. Additionally, the equilibrium vapor pressure of *trans*-ethylene- d_2 was reduced twice, allowing for the study of the lifetimes as a function of concentration, as shown in Figure 6. We were interested whether there is a variation in lifetime at different resonance conditions (i.e., different τ). Therefore, we conducted a generalized extreme studentized deviate (ESD) test [23] for each pressure. The generalized ESD test searches for up to k ($= 3$ used here) outliers and controls for the alternative hypotheses of $0 \dots k-1$ outliers. The test did not detect that any of the points were significantly outside of the variation. So, the experimental lifetimes at different τ show no significant difference.

The measurements of lifetime at a given pressure and resonance condition are aggregated and compared in Figure 6B and Table 1. The lifetimes can be compared to one another through a one-tailed t-test. Only the low pressure data were determined to be significantly different from the high and medium pressure data. The singlet lifetime was compared to the T_1 lifetime, where available and found to exceed it by a factor of ~ 30 . (For low pressure experiments, the short time component of the bi-exponential fit, T_{short} , was used to approximate the T_1 value). The ratio between the singlet lifetime and T_{short} is still substantial at the low pressure with a value of 15-20, and is likely a low estimate given the tendency of T_{short} to overestimate the T_1 value.

Figure 6: A & B) Experimental lifetime measurements at various interpulse delays (A: $\tau = 13.8$ ms; B: $\tau = 12.6$ ms) and number of pulses. Red circle markers indicate the high pressure (150 psi), blue diamonds the medium pressure (120 psi), and black squares the low pressure (29 psi) measurements. Error bars indicate the one σ confidence interval of the lifetime fit. C) Lifetime averaged across the number of pulses. Key is the same as in A, with error bars indicating the one σ confidence interval of the fit and the one σ standard deviation of the population added in quadrature.

	High Pressure (~ 150 psi, 270 mM)	Medium Pressure (~ 120 psi, 216 mM)	Low Pressure (~ 29 psi, 52 mM)
T_1	1.06 ± 0.02 s	1.53 ± 0.02 s	N/A
T_{short}	2.44 ± 0.44 s	2.94 ± 0.12 s	5.59 ± 0.32 s
$T_s @ \tau = 12.6$ ms	30.6 ± 12.3 s	38.9 ± 10.5 s	81.1 ± 18.3 s
$T_s @ \tau = 13.8$ ms	30.6 ± 10.7 s	44.2 ± 3.38 s	117.1 ± 9.80 s
$T_s / T_1 @ \tau = 12.6$ ms	28.9 ± 11.6	25.4 ± 6.87	$14.5 \pm 3.38^*$
$T_s / T_1 @ \tau = 13.8$ ms	28.9 ± 10.1	28.9 ± 2.24	$20.9 \pm 2.12^*$

Table 1: Lifetimes at various concentrations and resonance conditions. Errors indicate the one σ confidence interval of the fit and the one σ standard deviation of the population added in quadrature. The difference in lifetime was significant at the 5% level between the low pressure measurements and the higher pressure measurements. There was no significant difference between the high and medium pressure conditions. Also, there is no significant difference in lifetime between the resonance conditions. Significance testing was performed with a 1-tailed t-test. For the low pressure sample, an experimental T_1 measurement was not performed; however, the T_1 lifetime can be inferred from the short time component of the bi-exponential fit. T_{short} denotes this value and can be seen to be ~ 2 times longer than the T_1 lifetime. For both high and medium pressure, the singlet lifetime is nearly 30 times longer than its T_1 lifetime. For the low pressure, the T_s/T_1 ratio is calculated using T_{short} instead of T_1 (indicated by *) and is slightly lower than the other ratios, likely due to the overestimation of the T_1 time by T_{short} .

4. Conclusions

In conclusion, long lived singlet states have been observed on protons of deuterated ethylene gas. Substituting the *trans*-protons with deuterium, the singlet lifetime on protons is observed to be ~ 30 times longer than T_1 . We extended the theory of the AA'QQ' spin system to cover the case where J_{QQ} is a significant fraction of J_{AA} . The relaxation behavior is explored via Gaussian and SPINACH simulations of the spin system and found to be in agreement with

experimental work. This geometry suggests a range of other interesting target systems, such as ring structures with deuterium atoms on opposite sides of the ring (e.g. CHDR-CDHR) as motifs for storage of hyperpolarization possibly induced by parahydrogen hyperpolarization.

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Appendix

Both Gaussian09 and SPINACH NMR simulation parameters and options are listed. The Gaussian09 returned values include magnetic shielding tensors, atomic coordinates, *J*-coupling matrix and quadrupolar tensors, they are also listed below.

The parameters used in Gaussian09 simulation are:

```
nmr=(giao,spinspin, mixed, susceptibility) b3lyp/6-31G(df,3p)
scrf=(solvent=heptane)
geom=connectivity output=pickett
```

Since there is no hexane listed in the software, heptane is selected as solvent, which is closest to hexane.

Gaussian return magnetic shielding tensors:

```
SCF GIAO Magnetic shielding tensor (ppm):
 1 C Isotropic = 71.8336 Anisotropy = 155.8908
XX= -40.4870 YX= 0.0043 ZX= 0.0000
XY= 0.0060 YY= 80.2269 ZY= 0.0000
XZ= 0.0000 YZ= 0.0000 ZZ= 175.7607
Eigenvalues: -40.4870 80.2269 175.7607
 2 H Isotropic = 25.7240 Anisotropy = 4.4774
XX= 22.7972 YX= -1.1117 ZX= 0.0000
XY= 1.0778 YY= 28.7088 ZY= 0.0000
XZ= 0.0000 YZ= 0.0000 ZZ= 25.6658
Eigenvalues: 22.7972 25.6658 28.7089
 3 H Isotropic = 25.7239 Anisotropy = 4.4768
XX= 22.7971 YX= 1.1125 ZX= 0.0000
XY= -1.0769 YY= 28.7084 ZY= 0.0000
XZ= 0.0000 YZ= 0.0000 ZZ= 25.6662
Eigenvalues: 22.7971 25.6662 28.7084
 4 C Isotropic = 71.8336 Anisotropy = 155.8908
XX= -40.4870 YX= 0.0043 ZX= 0.0000
XY= 0.0060 YY= 80.2269 ZY= 0.0000
XZ= 0.0000 YZ= 0.0000 ZZ= 175.7607
Eigenvalues: -40.4870 80.2269 175.7607
 5 H Isotropic = 25.7240 Anisotropy = 4.4774
XX= 22.7972 YX= -1.1117 ZX= 0.0000
XY= 1.0778 YY= 28.7088 ZY= 0.0000
XZ= 0.0000 YZ= 0.0000 ZZ= 25.6658
Eigenvalues: 22.7972 25.6658 28.7089
 6 H Isotropic = 25.7239 Anisotropy = 4.4768
XX= 22.7971 YX= 1.1125 ZX= 0.0000
```


XY= -1.0769 YY= 28.7084 ZY= 0.0000
 XZ= 0.0000 YZ= 0.0000 ZZ= 25.6662
 Eigenvalues: 22.7971 25.6662 28.7084

Atomic coordinates:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	6	0	0.000000	0.666858	0.000000
2	1	0	0.931540	1.241471	0.000000
3	1	0	-0.931492	1.241524	0.000000
4	6	0	0.000000	-0.666858	0.000000
5	1	0	-0.931540	-1.241471	0.000000
6	1	0	0.931492	-1.241524	0.000000

J-coupling matrix:

Total nuclear spin-spin coupling J (Hz):

	1	2	3	4	5	6
1	0.000000D+00					
2	0.248910D+02	0.000000D+00				
3	0.162159D+03	0.351990D+00	0.000000D+00			
4	0.717805D+02	-0.188404D+00	-0.122546D+01	0.000000D+00		
5	-0.188404D+00	0.469915D+00	0.189002D+01	0.248910D+02	0.000000D+00	
6	-0.122546D+01	0.189002D+01	0.199412D+02	0.162159D+03	0.351990D+00	0.000000D+00

The quadrupolar tensors:

Nuclear quadrupole coupling constants [Chi] (MHz):

2 H(2)

aa = 0.0272 ba = 0.1389 ca = 0.0000
 ab = 0.1389 bb = 0.0716 cb = 0.0000
 ac = 0.0000 bc = 0.0000 cc = -0.0988

5 H(2)

aa = 0.0272 ba = 0.1389 ca = 0.0000
 ab = 0.1389 bb = 0.0716 cb = 0.0000
 ac = 0.0000 bc = 0.0000 cc = -0.0988

Some of parameters and options used for SPINACH NMR simulations are:

Relaxation theory is Bloch-Redfield-Wangsness: inter.relaxation = "redfield"

The superoperator is in laboratory frame: inter.rlx_keep = "labframe"

Rotational correlation time is 0.1×10^{-12} s: inter.tau_c = 0.1e-12

The basis set is Zeeman-Liouville: bas.formalism = "zeeman-liouv"