Accessing Long-Lived Disconnected Spin-$^1/_2$ Eigenstates through Spins > $^1/_2$

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S *Supporting Information*

ABSTRACT: Pairs of chemically equivalent (or nearly equivalent) spin-$^1/_2$ nuclei have been shown to create disconnected eigenstates that are very long-lived compared with the lifetime of pure magnetization (T_1). Here the classes of molecules known to have accessible long-lived states are extended to include those with chemically equivalent spin-$^1/_2$ nuclei accessed by coupling to nuclei with spin > $^1/_2$, in this case deuterium. At first, this appears surprising because the quadrupolar interactions present in nuclei with spin > $^1/_2$ are known to cause fast relaxation. Yet it is shown that scalar couplings between deuterium and carbon can guide population into and out of long-lived states, i.e., those immune from the dominant relaxation mechanisms. This implies that it may be practical to consider compounds with ^{13}C pairs directly bound to deuterium (or even ^{14}N) as candidates for storage of polarization. In addition, experiments show that simple deuteration of molecules with ^{13}C pairs at their natural abundance is sufficient for successful lifetime measurements.

Hyperpolarization is a promising method for imaging a wide variety of metabolic pathways in vivo. For instance, the conversion from pyruvate to lactate, useful for early-stage cancer detection, has been demonstrated in humans.[1] The biggest limitation of hyperpolarized magnetic resonance imaging (HP-MRI) is that the hyperpolarization decays back to thermal equilibrium at a rate given by the nuclear spin relaxation time T_1, which in solution is commonly seconds. These short relaxation times drastically limit the range of molecules and processes that can be studied. A powerful solution to the imaging time problem comes in the form of "disconnected eigenstates" with very long lifetimes, such as the singlet ($\alpha\beta - \beta\alpha$) between a single pair of inequivalent spins that can be maintained by going to low fields[2] or by spin locking.[3] Another state, one that can persist at any magnetic field without spin locking, is the state $(\alpha\beta - \beta\alpha)_{^{13}C}(\alpha\beta - \beta\alpha)_{^1H}$ between pairs of chemically equivalent spins.[4] The case of chemically equivalent spins is particularly interesting because high degrees of symmetry make the accessed states particularly long-lived. At first glance, it is surprising that these disconnected states are even accessible without a chemical shift difference; however, the out-of-pair J couplings break the magnetic equivalence and create a handle to access the disconnected eigenstates. Some of these disconnected eigen-

states have been shown to have exceedingly long lifetimes, in excess of hundreds of seconds or over 80 times T_1.[5,6]

Previous experimental work on chemically equivalent spins has demonstrated long-lived states of AA'XX' (e.g., diacetylene; Figure 1) and AA'X$_2$X$_2$' systems consisting entirely of spin-$^1/_2$
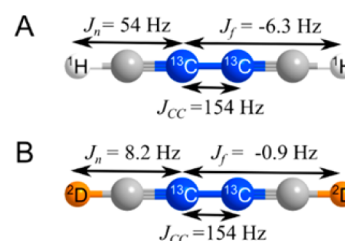

Figure 1. (A) Protonated diacetylene and the involved J-coupling network. (B) Deuterated diacetylene; the near and far heteronuclear J couplings are reduced by a factor of 6.5 compared with the near and far heteronuclear J couplings in (A).

nuclei.[4,5,7,8] In these cases, the singlet–singlet state is only close to an eigenstate if $J_{CH} \ll J_{CC}$; otherwise, spin locking or decoupling is required to preserve the state (with associated penalties in power deposition). Since one-bond CC couplings ($^1J_{CC}$) are in the range of 30−200 Hz,[9] this places some restrictions on the applicability; specifically, protons directly bound to a pair of ^{13}C nuclei have $^1J_{CH} \sim 150$ Hz, and therefore, all demonstrations have avoided that case. Deuterium substitution might be useful, as it reduces the J coupling by a factor of 6.5 (the ratio of the gyromagnetic ratios γ_H/γ_H), and directly bonded deuterium might then support a long-lived state. Although direct dipole−dipole relaxation of the ^{13}C pair is prohibited by symmetry, there may exist intermolecular effects that break this symmetry and allow relaxation. This would be reduced for the reduced dipole moment from deuterium. However, nuclei with spin > $^1/_2$ experience quadrupolar relaxation, which is absent for spin-$^1/_2$ nuclei, and therefore analysis is necessary.

Here, long-lived states are demonstrated to exist in deuterated 2,3-^{13}C diacetylene (Figure 1B) and deuterated diphenylacetylene (DPA) labeled at the acetylenic carbons. A carbon-only pulse sequence[6] [Figure S1 in the Supporting Information (SI)] was applied to deuterated diacetylene (but with ^{13}C at its natural abundance) in a 700 MHz NMR spectrometer. A long-lived state was observed from the
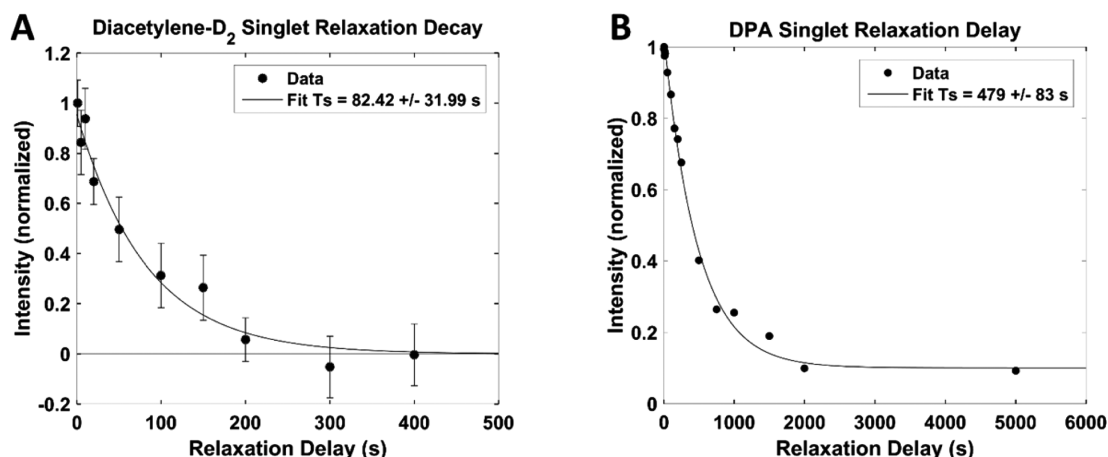
Figure 2. (A) Decay curve for deuterated diacetylene. Each data point is an average of 64−136 acquisitions. The fit shown is a single-exponential fit to the data points at delays longer than 10 s to avoid any rapid triplet relaxation. (B) Singlet decay curve for deuterated and labeled diphenylacetylene (DPA) acquired without decoupling. For both signals, error bars represent $\pm 2\sigma$ (95%) variance in the noise (obscured by marker in (B)), and the fit errors are reported at the 95% confidence interval.

statistically labeled 2,3-$^{13}C_2$ fraction (only 0.01% of the molecules). In addition, deuterated and ^{13}C-labeled DPA was synthesized and studied in a 360 MHz spectrometer with a much better signal-to-noise ratio. The singlet states supported by protonated DPA were previously studied, as the DPA moiety can appear in antibiotic and anticancer drugs.[5]

The results are shown in Figure 2. A lifetime of $T_S = 83 \pm 30$ s was measured; this is 5 times the ^{13}C T_1 of 16.7 ± 0.9 s, ~160 times the ^2H T_1 of 0.5 ± 0.2 s, and ~2 times the reported singlet relaxation time for protonated diacetylene ($T_S = 49 \pm 23$ s).[10] The DPA sample was observed to have a deuterated singlet lifetime of $T_S = 479 \pm 83$ s, nearly 1.7 times the reported protonated singlet lifetime ($T_S = 274 \pm 6.1$ s).[5]

In the following, the form of the deuterium−carbon long-lived states of AA′QQ′ systems, where A is spin-$^1/_2$ and Q is spin-1, is derived and some nonintuitive results are noted. For example, the long-lived state in the AA′XX′ system is the spin-0 combination of each pair of spins (singlet$_A$−singlet$_X$); the AA′QQ′ system also has a spin-0 combination for each spin pair, but that is not the most efficient or robust way to pump the long-lived states. In addition, the lifetime of the shorter-lived species (hydrogen in the AA′XX′ systems, deuterium in the AA′QQ′ system) plays almost no role. Although the spin-1 nucleus studied here is deuterium, systems containing ^{14}N may also prove useful. Overall, this implies a significantly wider range of labeled molecular targets that might be useful for long-time hyperpolarized imaging.

To understand why this works, consider the AA′XX′ case. Previous work has shown that the disconnected eigenstates for AA′XX′ systems can be accessed with two fundamentally different pulse sequences in the high-field limit.[4,7] The first method is an adaption of the magnetization-to-singlet (M2S) sequence pioneered by Levitt and co-workers for a pair of spin-$^1/_2$ nuclei with a small chemical shift difference.[11,12] The interaction Hamiltonian of the coupled spin pairs of the four-spin system is

$$H_{\text{interaction}} = 2\pi J_{AA'}\mathbf{S}_1\cdot\mathbf{S}_2 + 2\pi J_{XX'}\mathbf{I}_1\cdot\mathbf{I}_2$$
$$+ 2\pi J_n(I_{1z}S_{1z} + I_{2z}S_{2z}) + 2\pi J_f(I_{1z}S_{2z} + I_{2z}S_{1z})$$
$$= 2\pi J_{AA'}\mathbf{S}_1\cdot\mathbf{S}_2 + 2\pi J_{XX'}\mathbf{I}_1\cdot\mathbf{I}_2 + \pi(J_n + J_f)$$
$$(I_{1z} + I_{2z})(S_{1z} + S_{2z}) + \pi(J_n - J_f)(I_{1z} - I_{2z})$$
$$(S_{1z} - S_{2z})$$
$$= H_{AA'} + H_{XX'} + H_{\Sigma J} + H_{\Delta J} \qquad (1)$$

where, by symmetry, $J_n = J_{AX} = J_{A'X'}$ and $J_f = J_{AX'} = J_{A'X}$. Here the states of A (typically the species with the longer-lived singlet, such as ^{13}C) and X (typically protons) are labeled in the singlet−triplet basis: $S = (\alpha\beta - \alpha\beta)/\sqrt{2}$, $T_{+1} = \alpha\alpha$, $T_0 = (\alpha\beta + \alpha\beta)/\sqrt{2}$, $T_{-1} = \beta\beta$. As has been shown previously,[4] the Hamiltonian in eq 1 is not diagonal in this basis. Instead, the Hamiltonian is block-diagonalized into two two-level systems joining triplets and singlets; one block joins the states $S^A S^X$ and $T_0^A T_0^X$, and another block joins $S^A T_0^X$ and $T_0^A S^X$. The elements that connect these states are those that break the magnetic equivalence and are proportional to $\Delta J = J_n - J_f$, the difference between the two A−X couplings. For example, the singlet−singlet and triplet−triplet block is

$$
\begin{array}{cc}
& \begin{array}{cc} T_0^A T_0^X & \qquad\qquad S^A S^X \end{array} \\
\begin{array}{c} T_0^A T_0^X \\ S^A S^X \end{array} &
\begin{pmatrix}
\pi(J_{AA} + J_{XX}) & -\pi\Delta J \\
-\pi\Delta J & -\pi(J_{AA} + J_{XX})
\end{pmatrix}
\end{array}
\qquad (2)
$$

If $\Delta J \ll J_{AA} + J_{XX}$, then the singlet−singlet and triplet−triplet states are nearly eigenstates, but transitions between the $T_0^A T_0^X$ and $S^A S^X$ states can be made by compensating for the diagonal terms ($J_{AA} + J_{XX}$). M2S accomplishes this with a train of π pulses applied on one nuclear species (^{13}C or ^1H) with spacing $\tau = 1/\{2[(J_{AA} + J_{XX})^2 + (\Delta J)^2]^{1/2}\}$.[4,11] In an interaction representation, such a sequence modulates ΔJ at the resonance frequency given by the J couplings and transfers population from the triplet to the singlet, much as a radiofrequency (RF) pulse modulated at the Larmor resonance frequency transfers population in a conventional NMR pulse.[4]

The second method, spin-lock-induced crossing (SLIC), was also introduced as a procedure for generating the singlet state on a single pair of inequivalent spin-$\frac{1}{2}$ systems.[13] In this case, continuous-wave RF irradiation of amplitude equal to $2\pi(J_{AA} + J_{XX})$ is applied, effectively eliminating the diagonal term. As in M2S, it is the out-of-pair difference coupling, ΔJ, that drives population between the triplet and singlet states.[7] The extension of the M2S sequence for the spin-1 system is discussed below, and the extension to the SLIC sequence is briefly described. A full discussion involving the SLIC basis set may be found in the SI.

In the AA′QQ′ system, the interaction Hamiltonian is essentially the same, in operator form, as that of AA′XX′ in eq 1:

$$
\begin{aligned}
H_{\text{interaction}} &= H_{AA} + H_{QQ} + H_{AQ} \\
&= 2\pi J_{AA}\mathbf{S}_1 \cdot \mathbf{S}_2 + 2\pi J_{QQ}\mathbf{I}_1 \cdot \mathbf{I}_2 \\
&\quad + 2\pi J_n(I_{1z}S_{1z} + I_{2z}S_{2z}) + 2\pi J_f(I_{1z}S_{2z} + I_{2z}S_{1z})
\end{aligned}
\tag{3}
$$

However, for AA′QQ′ there are 36 energy levels ($2 \times 2 \times 3 \times 3$), which makes the problem appear complicated, but symmetry reductions help dramatically, and again it will be shown that ΔJ drives the population transfer in the spin-1 systems. The states of carbon are written in the singlet−triplet basis (S, T_{+1}, T_0, T_{-1}). The states of the individual deuteriums are represented by the letters P, Z, and M (for plus, zero, and minus), which correspond to $m_z = +1$, 0, and -1, respectively. The two spin-1 nuclei can be written in their combined $|F, M\rangle$ basis (where F represents the total angular momentum and M its magnetic z component), in which they form a spin-2 manifold,

$$
\begin{aligned}
|2, 2\rangle &= \text{PP} \\
|2, 1\rangle &= (\text{PZ} + \text{ZP})/\sqrt{2} \\
|2, 0\rangle &= (\text{PM} + 2\cdot\text{ZZ} + \text{MP})/\sqrt{6} \\
|2, -1\rangle &= (\text{MZ} + \text{ZM})/\sqrt{2} \\
|2, -2\rangle &= \text{MM}
\end{aligned}
\tag{4}
$$

a spin-1 manifold,

$$
\begin{aligned}
|1, 1\rangle &= (\text{PZ} - \text{ZP})/\sqrt{2} \\
|1, 0\rangle &= (\text{PM} - \text{MP})/\sqrt{2} \\
|1, -1\rangle &= (\text{MZ} - \text{ZM})/\sqrt{2}
\end{aligned}
\tag{5}
$$

and a spin-0 manifold,

$$
|0, 0\rangle = (\text{PM} - \text{ZZ} + \text{MP})/\sqrt{3}
\tag{6}
$$

The full basis set is formed by combining these deuterium states with the singlet−triplet states of the ^{13}C pair. Upon examination of these states under the interaction Hamiltonian given in eq 3, H_{QQ} may be removed since the deuterium−deuterium coupling is weak across the five-bond connection in diacetylene. It should be noted that even for three-bond couplings, protons have ^3J values of $\sim$1−20 Hz, which are reduced by a factor of 42 ($=6.5^2$) for deuterium. (The consequences of a nonzero J_{QQ} are discussed in the SI.) Of the remaining terms, H_{AA} and $H_{\Sigma J}$ act along the diagonal, and only $H_{\Delta J}$ appears off-diagonal. In particular, there are six pairs of

states that have nonzero elements of $H_{\Delta J}$, yielding six two-level systems, which are shown in Table 1. The matrix elements of the first two-level system in Table 1 are

$$
\begin{array}{c}
\quad\quad\quad |2, 1\rangle T_0 \quad\; |1, 1\rangle S \\
\begin{array}{cc}
|2, 1\rangle T_0 \\
|1, 1\rangle S
\end{array}
\begin{pmatrix}
0 & \pi\Delta J \\
\pi\Delta J & -2\pi J_{AA}
\end{pmatrix}
\end{array}
\tag{7}
$$

Table 1. Two-Level Systems in the AA′QQ′ System, Where Q and Q′ Are Spin-1 Nuclei

two-level system		M_D
$|2, 1\rangle T_0$	$|1, 1\rangle S$	+1
$[(|2, 0\rangle + \sqrt{2}|0, 0\rangle)/\sqrt{3}]T_0$	$|1, 0\rangle S$	0
$|2, -1\rangle T_0$	$|1, -1\rangle S$	−1
$|1, 1\rangle T_0$	$|2, 1\rangle S$	+1
$|1, 0\rangle T_0$	$[(|2, 0\rangle + \sqrt{2}|0, 0\rangle)/\sqrt{3}]S$	0
$|1, -1\rangle T_0$	$|2, -1\rangle S$	−1

The off-diagonal elements, $\pi\Delta J$, drive transitions between the singlet and triplet states. The pairs of states with $M_D \pm 1$ from Table 1 have identical matrix elements. On the other hand, the states with total deuterium spin projection $M_D = 0$ form a two-level system with doubled off-diagonal elements. The matrix elements from the first two-level system with $M_D = 0$ are

$$
\begin{array}{c}
\quad\quad \frac{1}{\sqrt{3}}(|2, 0\rangle + \sqrt{2}|0, 0\rangle)T_0 \quad |1, 0\rangle S \\
\begin{array}{c}
\frac{1}{\sqrt{3}}(|2, 0\rangle + \sqrt{2}|0, 0\rangle)T_0 \\[1ex]
|1, 0\rangle S
\end{array}
\begin{pmatrix}
0 & 2\pi\Delta J \\
2\pi\Delta J & -2\pi J_{AA}
\end{pmatrix}
\end{array}
\tag{8}
$$

This system will evolve at twice the rate of the $M_D \pm 1$ two-level systems. (The SI includes a discussion of the mixing of the $|2,0\rangle$ and $|0,0\rangle$ states, which becomes important when $J_{QQ} \neq 0$.)

The description above is in the basis that makes the effects of the M2S sequence tractable. The SLIC experiments follow a similar construction. For brevity, the changes and results are outlined here, while a full discussion may be found in the SI. For an experiment performed with only carbon pulses, the spin-$\frac{1}{2}$ nuclei are now written in the X basis (the eigenbasis of $S_{1x} + S_{2x}$, not to be confused with the X labels of the spin system). The spin-1 basis remains the same. The results for the AA′QQ′ system are that the two-level systems with $M_D = \pm 1$ become four-level systems, and the off-diagonal elements driving the triplet−singlet transition appear as $\pi J/\sqrt{2}$. The two-level systems with $M_D = 0$ also mix into four-level systems that still have a transition frequency twice that of the others ($\pi J\sqrt{2}$) and undergo the triplet to singlet transition in half the time of the others. For both M2S and SLIC, eight of the 36 states (two-ninths) in a C_2D_2 spin system are singlet−triplet-coupled, and four states are coupled at the double frequency. Simulations performed with SPINACH[14] showed that 30% of the transverse magnetization can be transferred to the singlet state in deuterated diacetylene, compared with 33% for the protonated compound. For DPA, these values are 24% (deuterated) and 33% (protonated). These numbers are broadly consistent with those observed experimentally (e.g., 20% SLIC efficiency was measured in deuterated DPA).

Finally, consider the dominant relaxation effects. The spin-0 "singlet" state of two isolated spin-$\frac{1}{2}$ nuclei has no dipole-allowed transitions to any other state. This makes it immune to relaxation from mechanisms that do not break the symmetry. However, the spin-0 state from two spin-1 nuclei is not isolated in the same way. It is also protected from dipolar relaxation, but the first-order quadrupolar interaction $[H_{\text{quadrupolar}} \propto \sum_i (3I_{z,i}^2 - |\mathbf{I}_i|^2)]$, which is present in nuclei with spin $> \frac{1}{2}$, affects P and M differently than Z; thus, the effect of a fluctuating electric field will be to mix $|0, 0\rangle$ and $|2, 0\rangle$, and the latter has allowed dipolar transitions. Therefore, quadrupolar relaxation is expected to be an issue with the deuterium states. Despite this, the systems studied here have a long-lived state for which the extended lifetime depends on the carbon character of the disconnected state, not on the proton or deuterium component. If the proton or deuterium component relaxes to equilibrium, the carbon singlet character can be preserved, and a significant fraction can be recalled into observable magnetization. In all of these systems, it is best to view the proton or deuterium as essentially an "auxiliary spin" that provides the couplings needed to guide the carbon into the singlet but is not itself particularly long-lived. For similar reasons, spin-1 ^{14}N may well be useful when combined with carbon or proton coherences. For example, the SABRE method for hyperpolarization[15−17] generally uses molecules with this AA′XX′ motif, where A and A′ are para-hydrogen and X and X′ (or Q and Q′) are spins on molecules bound to an iridium complex (e.g., pyridine).

In conclusion, it is possible to generate long-lived singlet states on chemically equivalent spin-$\frac{1}{2}$ pairs by coupling to a pair of chemically equivalent spin-1 pairs, which breaks the magnetic equivalence of both pairs. At first glance, it may be surprising that long-lived states are obtained since even the disconnected (spin-0) state of the combined spin-1 nuclei is exposed to quadrupolar relaxation. Nevertheless, AA′QQ′ systems can store polarization for times much longer than T_1 in a singlet on the spin-$\frac{1}{2}$ nuclei. This allows singlet states in systems that were previously unattainable, such as chemically equivalent carbons directly bonded to deuterium: In directly bonded $^{13}C_2{}^1H_2$ systems, the ^{13}C−^{1}H J coupling is often stronger than the ^{13}C−^{13}C J coupling, preventing a stable singlet state. The weaker deuterium coupling could support the singlet states in such one-bond coupled systems, extending the range of molecules of promise for long-lived hyperpolarized MR. Lastly, by extending long-lived singlet states to integer spin nuclei, molecules containing ^{14}N may also be considered as coupling partners to access long-lived singlets without expensive and time-consuming ^{15}N labeling schemes.

ASSOCIATED CONTENT

Supporting Information

Experimental details (equipment, pulse sequence, and synthesis) and a full treatment of the spin-1 system (including the J_{DD} terms). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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