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Storage of Hydrogen Spin Polarization in Long-Lived $^{13}C_2$ Singlet Order and Implications for Hyperpolarized Magnetic Resonance Imaging

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Supporting Information

ABSTRACT: Hyperpolarized magnetic resonance imaging (MRI) is a powerful technique enabling real-time monitoring of metabolites at concentration levels not accessible by standard MRI techniques. A considerable challenge this technique faces is the T_1 decay of the hyperpolarization upon injection into the system under study. Here we show that $A_nA'_nXX'$ spin systems such as $^{13}C_2$-1,2-diphenylacetylene ($^{13}C_2$-DPA) sustain long-lived polarization for both ^{13}C and ^{1}H spins with decay constants of almost 4.5 min at high magnetic fields of up to 16.44 T without spin-locking; the T_1 of proton polarization is only 3.8 s. Therefore, storage of the proton polarization in a $^{13}C_2$-singlet state causes a 69-fold extension of the spin lifetime. Notably, this extension is demonstrated with proton-only pulse sequences, which can be readily implemented on standard clinical scanners.

In almost all preclinical and clinical magnetic resonance imaging (MRI) experiments, the acquired signal originates from the hydrogen nuclei in water. This is because in typical MRI experiments polarization levels are on the order of 10^{-5}, and only signals from molecules at high concentrations such as water can easily be detected. To overcome this limitation, hyperpolarization techniques to enhance magnetization by more than 10,000-fold have been developed.[1−3] They have a wide range of applications, including *in vivo* imaging of molecular markers at millimolar concentrations. However, the generality of this method is hindered by the T_1 relaxation time, which is on the order of a few seconds for most molecules.[4] In this context, the long-lived singlet state between a pair of strongly coupled spin-1/2 nuclei (mostly, ^{1}H, ^{13}C, or ^{15}N) has drawn considerable attention,[5−8] since hyperpolarization stored as singlet order might enable tracking of *in vivo* imaging agents and their metabolism on time scales of minutes.

Singlet states located on low-γ spin pairs such as $^{13}C_2$ and $^{15}N_2$ have been shown to sustain long-lived signal up to tens of minutes.[9,10] However, for hyperpolarized imaging, the signal-to-noise ratio (SNR) can be estimated to scale linearly with γ in body-noise-dominated MRI experiments, and with $\gamma^{7/4}$ in coil-noise-dominated NMR experiments.[11,12] Although the $^{1}H_2$ singlet state can also extend ^{1}H magnetization lifetime,[7] the absolute singlet lifetime T_S is generally relatively short (an exception is presented by Buljubasich et al.,[13] where the singlet has to be unlocked by shuttling out of the high-field magnet to a specific field of 0.1 T) due to residual relaxation effects that are proportional to γ, such as intermolecular dipole−dipole interactions. Ideally, hyperpolarized signal would be stored in $^{13}C_2/^{15}N_2$ singlet states but detected later on nearby protons. This might be conceivable with conventional polarization transfer techniques (such as INEPT), but multiple bond X−H scalar couplings in these systems are usually too small, giving rise to significant T_2 loss during polarization transfer, and protons directly bonded to ^{13}C are well known to drastically shorten the polarization lifetime.

In the present study, we demonstrate convenient polarization interconversion between a $^{13}C_2$-singlet state and nearby ^{1}H magnetization with a modification to a previously demonstrated "magnetization-to-singlet" pulse sequence (Figure 1a,b).[14,15]

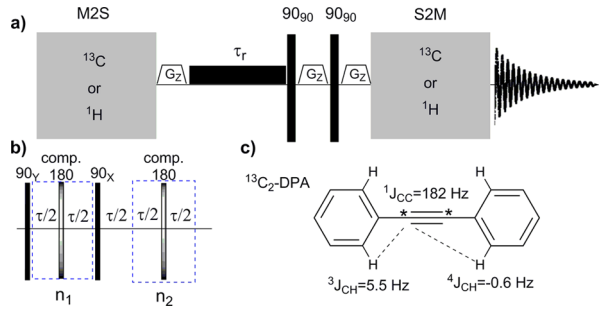

Figure 1. (a) The MSM sequence composed of M2S and S2M which are separated by the relaxation delay τ_r and a combination of gradients (G_Z) and 90° pulses to suppress thermal artifacts. S2M is the inverse of M2S. For $^{13}C_2$-DPA, M2S and S2M can also be applied on ^{1}H. (b) The "magnetization-to-singlet" (M2S) sequence which converts bulk magnetization to singlet order. The sequence is composed of two 180° pulse trains where $n_1 = 2n_2$. See SI for details. (c) $^{13}C_2$-diphenylacetylene ($^{13}C_2$-DPA) with the *J*-coupling constants that determine n_1, n_2, and τ.

This sequence was first introduced to excite a pair of slightly inequivalent spins,[14,16] but we recently showed that a similar approach works with chemically equivalent spins, as long as they are asymmetrically coupled to other out-of-pair spins.[15] In this first demonstration for the $CC'H_2H_2'$ spin system in diethyl oxalate,[15] the interconversion between ^{13}C magnetization and $^{13}C_2$-singlet order was shown. An important difference between chemically inequivalent and chemically

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Table 1. Summary of Measured and Simulated T_1 and Singlet Relaxation Time (T_S) at Varied Field Strengths[a]

field strength, B_0/T	experiment type	theoretical $T_1(^{13}C)$/s	theoretical T_s/s	experimental $T_1(^{13}C; ^1H)$ /s	experimental T_S/s
8.45	MSM(^{13}C)	12.2	274.7 ± 6.1	^{13}C, 13.9; ^1H, 3.7	288.4 ± 3.7
8.45	MSM(^{13}C) with ^1H decoupling	–	274.7 ± 6.1	–	267.1 ± 5.4
8.45	M2S(^1H), S2M(^{13}C)	–	273.2 ± 7.5	–	282.7 ± 3.8
16.44	MSM(^{13}C)	4.6	252.0 ± 5.0	^{13}C, 4.9; ^1H, 3.8	244.7 ± 1.4
16.44	M2S(^{13}C), S2M(^1H)	–	276.7 ± 8.5	–	223 ± 9.1
16.44	MSM(^1H)	–	252.5 ± 15.3	–	261.7 ± 7.3
1.5	MSM(^{13}C)	29.1	309.3 ± 8.2	–	–

[a]Detection always occurs on the S2M channel; for instance, S2M(^1H) detects on the proton channel. Errors are obtained during the fitting procedure of the data which also arise for the theoretical values because of oscillations generated by coherent effects in the beginning of the decay traces as apparent from Figure S3 and explained in more detail in the SI.

equivalent spin systems is that, in the latter case, with spin systems such as $CC'H_2H_2'$, the sequences can be selectively pulsed on ^{13}C or ^1H. In the current study, this is the key to explore the previously untapped ^1H magnetization, transferring it into $^{13}C_2$-singlet polarization. Therefore, either ^{13}C or ^1H magnetization can be hyperpolarized and converted into $^{13}C_2$-singlet polarization. Likewise, the $^{13}C_2$-singlet polarization can be converted back into either ^{13}C or ^1H magnetization for detection.

The spin system we use here is $^{13}C_2$-1,2-diphenylacetylene (^{13}C-DPA, Figure 1c). Recently, molecules containing $^{13}C_2$-acetylene substructures have been reported to sustain long-lived carbon singlet states.[10] In this moiety the scalar coupling between the ^{13}C spins is consistently large (~180 Hz), which can suppress the singlet−triplet mixing caused by either small chemical shift differences or small out-of-pair J-coupling differences. Moreover, the linear configuration of $^{13}C_2$-acetylene can result in highly correlated chemical shift anisotropy (CSA) perturbation on the two ^{13}C spins[17] and therefore largely extended singlet lifetimes compared to ^{13}C-T_1 were observed.[10,18] It is worth noting that in the previous study samples were degassed, nearby protons were deuterated, and polarization could only be transferred from the singlet-bearing spins (^{13}C) to the $^{13}C_2$-singlet state.

In contrast, in our study with $^{13}C_2$-DPA, the sample is dissolved in $CDCl_3$ without degassing, and the phenyl groups are not deuterated. The small out-of-pair J-couplings ($^3J_{CH}$ and $^4J_{CH}$ in Figure 1c) between ^{13}C and the closest aromatic protons provide access to the $^{13}C_2$-singlet state. More importantly, magnetization from the aromatic protons can be transferred into $^{13}C_2$-singlet polarization and vice versa. We show that this enables proton-only experiments with observed relaxation lifetimes of almost 4.5 min at 16.44 T despite the very short proton T_1 times of 3.8 s at the same field strength. All relaxation lifetime measurements and theoretical predictions are summarized in Table 1.

For $^{13}C_2$-DPA, the difference of the J-couplings of either ^{13}C spin to the same aromatic proton, $\Delta J_{CH} = {}^3J_{CH} - {}^4J_{CH}$, is around 6.1 Hz, much smaller than J_{CC} (182 Hz), resulting in small singlet−triplet mixing. As described previously[15] and detailed in the Supporting Information (SI), the MSM sequence parameters can be adjusted to resonate with this mixing effect and thus achieve interconversion between ^{13}C bulk magnetization and $^{13}C_2$-singlet state population. In a field of 8.45 T, the singlet state lifetime T_S is measured to be 288.4 ± 3.7 s (Figure 2, black curve). This singlet state relaxation rate has contributions from multiple mechanisms, including the coherent mixing between singlet and triplet states. Previous studies have shown that the relaxation rate due to the coherent

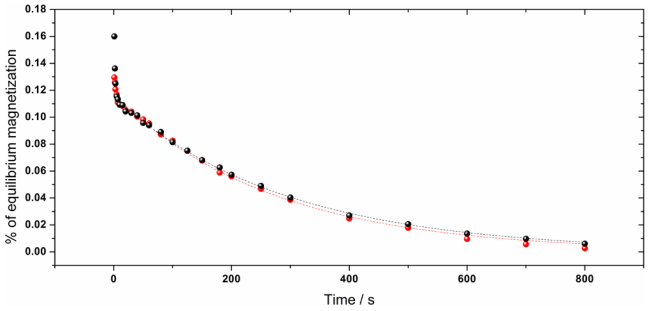

Figure 2. Observed $^{13}C_2$-DPA singlet state relaxation at 8.45 T with the MSM sequence. The singlet relaxation is preceded by a fast triplet relaxation on the order of T_1 (12 s), and after ~15 s a single exponential decay (singlet lifetime, T_S) of 288.4 ± 3.7 s is observed without ^1H decoupling (black) and a T_S of 267.1 ± 5.4 is observed with ^1H decoupling during the delay between M2S and S2M (red). Signal is normalized against equilibrium magnetization obtained by a separate 90°-acquire experiment.

effect is proportional to $(\Delta J_{CH}/J_{CC})$;[28] thus, we expect a trivial effect for $^{13}C_2$-DPA. This is verified by a second measurement (Figure 2, red curve), where the coherent mixing due to ΔJ_{CH} is suppressed by ^1H decoupling during the relaxation delay (τ_r in Figure 1a). Indeed, no significant change of T_S was observed; in fact, a slightly shorter lifetime was measured, which may be due to heating effects from the extended irradiation during ^1H decoupling.

Because the resonance condition of MSM sequence is independent of field strength, the same measurement can be conveniently transferred to a different field strength (16.44 T). This can help distinguish the major relaxation effects (dipole−dipole interactions between the ^{13}C spins is expected to be field independent within the extreme narrowing regime,[19−21] whereas CSA relaxation has a quadratic dependence on B_0[21]). For $^{13}C_2$-DPA, the nearly doubled magnetic field reduces ^{13}C T_1 to about 35% of its original value, whereas T_S is only reduced to about 90% of its original value (compare row 1 and 4 in Table 1). Ignoring any other relaxation effects, the relative contribution of CSA vs dipolar interactions can be estimated. CSA contributes 66% to the ^{13}C T_1 relaxation at 8.45 T and 88% at 16.44 T, whereas the relative CSA contribution to singlet-state relaxation is only 4% at 8.45 T and 14% at 16.44 T, confirming that the CSA at the ^{13}C sites must be highly correlated. On the other hand, CSA has minimum relaxation effects on ^1H; thus, ^1H-T_1 remains unchanged. The theoretical predictions[22] of relaxation lifetimes agree well with experimental results at 8.45 and 16.44 T (Table 1 and Figure S3). This gives us confidence in a theoretical extrapolation by

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simulation down to a field of 1.5 T (Table 1). The combination of lower field and hyperpolarization is especially appealing since SNR in hyperpolarized MRI experiments is generally field independent. In addition, in cases of slight chemical inequivalence, larger chemical shift differences can be tolerated which satisfy the near equivalent condition, so the geometrical constraints are relaxed.

As is shown in Figure 2, around 12% of the total ^{13}C magnetization has been converted into $^{13}C_2$-singlet state population, and therefore has an extended lifetime. This conversion efficiency is generally lower in chemically equivalent cases than in the case of a single pair of inequivalent ^{13}C spins.

However, for chemically equivalent $A_nA'_nXX'$ spin systems such as contained in $^{13}C_2$-DPA, another opportunity arises. Proton magnetization can be used by applying the M2S part of the pulse sequence on the proton resonant channel as demonstrated in Figure 3. In so doing the initial proton

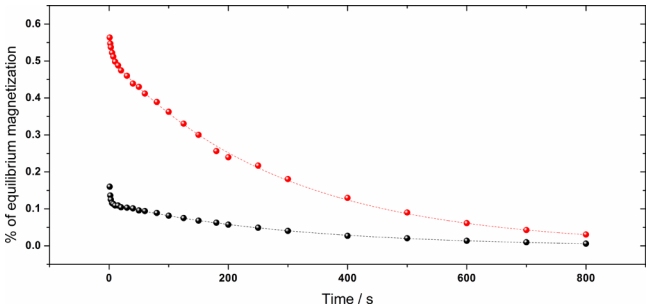

Figure 3. Observed $^{13}C_2$-DPA singlet state relaxation at 8.45 T. A T_S of 288.4 ± 3.7 s is observed (black, the same data as in Figure 2). This is compared to a singlet state relaxation measurement where the M2S part of the MSM sequence has been applied at the ^1H resonance frequency instead of the ^{13}C resonance frequency (red), resulting in an enhancement of the acquired signal of $\gamma_{^1H}/\gamma_{^{13}C} \approx 4$. For the singlet state relaxation using the ^1H polarization, a T_S of 282.7 ± 3.8 s is observed. The small discrepancy in the relaxation times is likely due to subtle differences in the contributions of thermal signal in the two measurements.

polarization that is larger by nearly a factor of $\gamma_{^1H}/\gamma_{^{13}C}$ can be transferred into the long-lived $^{13}C_2$-singlet state (Figure 3 demonstrates a signal enhancement of $\gamma_{^1H}/\gamma_{^{13}C} \approx 4$). This is because the M2S sequence, resonant with ΔJ_{CH}, can convert ^1H magnetization into the $^{13}C_2$-singlet, following a very similar pathway as that of ^{13}C magnetization.[15] In contrast, it is not possible to pursue this strategy if only one pair of slightly inequivalent spins[14] is used. Another intriguing possibility is to convert $^{13}C_2$-singlet polarization, created from initial ^1H magnetization, back into proton magnetization for detection. This can be done with pulses exclusively on the ^1H channel; therefore, this could be readily implemented on standard clinical imagers without a ^{13}C channel. A demonstration of this proton-only MSM experiment is shown in Figure 4a. A relaxation lifetime of 262 ± 7 s is observed at 16.44 T, where ^1H-T_1 is merely 3.8 s.

For a constant fractional hyperpolarization (e.g., 10% carbon hyperpolarization or 10% proton hyperpolarization) converted into the singlet, the signal is identical starting from either nucleus; however, the detection frequency out of the singlet gives SNR proportional to γ or $\gamma^{7/4}$ as mentioned earlier so detection of ^1H signal is certainly favorable (x4−x11 SNR for ^{13}C, x10−x56 for ^{15}N). Accordingly, M2S(X)−S2M(^1H) is a

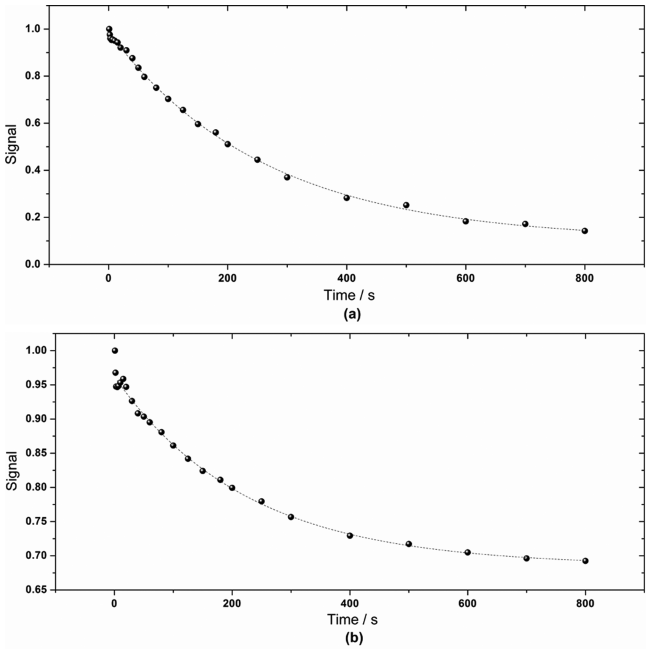

Figure 4. $^{13}C_2$-DPA singlet state relaxation at 16.44 T observed on the ^1H channel. (a) M2S and S2M applied on the ^1H channel such that the ^{13}C channel remains entirely unused. A T_S of 261 ± 7 s is observed. (b) M2S applied on the ^{13}C channel and S2M applied on the ^1H channel. A T_S of 223 ± 9 s is observed.

useful strategy, and Figure 4b demonstrates the singlet state relaxation after such an experiment. This long-lived ^1H signal originated from ^{13}C thermal polarization is small thus we observe residual ^1H thermal polarization in this experiment (note the ∼0.7 baseline offset in Figure 4b). This may also explain the smaller T_S (223 s) obtained from this measurement. On the other hand, recent studies have shown a higher polarization level can be achieved with ^1H hyperpolarization within a much shorter time compared with polarization buildup of ^{13}C or ^{15}N;[23,24] therefore, converting ^1H polarization into $^{13}C_2$-singlet polarization may also be beneficial. Transfer of ^1H magnetization from the hyperpolarizer to the imager may cause a significant signal loss due to short ^1H T_1, but this might be resolved by converting ^1H magnetization into $^{13}C_2$-singlet polarization within the hyperpolarizer.

Last, we want to point out that $^{13}C_2$-DPA, with a long-lived hyperpolarized signal, may potentially serve as a desirable labeling group. The chemical installation of 1,2-diphenylacetylene can be achieved modularly and readily.[25] Such an internal diarylacetylene functionality is chemically[26] and metabolically stable.[27] For example, diarylacetylene moieties are embedded in certain antibiotic drugs.[28,29] These are LpxC inhibitors such as CHIR-090.[28] Additionally, efforts are underway to synthesize drugs structurally related to suberolyanilide hydroxamic acid,[30] an FDA-approved anticancer drug sold under the trade name Vorinostat. It appears that such derivatives of $^{13}C_2$-DPA are easily synthesized by appropriate aromatic substitutions giving access to a wide range of functional groups that can be used to link to molecules with biological activity without severely disturbing the singlet-bearing spin system.[31]

In summary, we have shown the M2S−S2M sequence can be applied selectively on either ^{13}C frequency or ^1H frequency, leading to a 69-fold lifetime extension (∼4.5 min) for ^1H magnetization. This is especially significant given the relative

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proximity of the aromatic protons to the singlet bearing ^{13}C spins. The convenient polarization transfer between $^{13}C/^{1}H$ magnetization and $^{13}C_2$-singlet state population can further improve the SNR in hyperpolarized MRI/NMR experiments. For lower γ nuclei such as $^{15}N_2$-singlet state, this will be even more significant. On the other hand, MSM sequence entirely resonant with the ^{1}H frequency is very promising given the faster and larger absolute polarization levels achieved with ^{1}H hyperpolarization. Even the potentially large water and fat background is expected to be well separated from the signal of the aromatic protons. In such case, an experiment signal would be prepared and detected on ^{1}H (high γ nucleus) while it is stored on the $^{13}C_2/^{15}N_2$-singlet state (low γ nucleus) to diminish relaxation effects. Such an experiment does not even require heteronuclear capabilities on the spectrometer/imager and is readily achievable on standard clinical imagers.

■ ASSOCIATED CONTENT

Supporting Information

Detailed pulse sequence parameters, synthetic routes, sample ^{13}C spectra, and description of the simulation methods. This material is available free of charge via the Internet at http://pubs.acs.org

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‡Y.F. and T.T. contributed equally.

Notes

The authors declare no competing financial interest.

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