Polychromatic Excitation of Delocalized Long-Lived Proton Spin States in Aliphatic Chains

Anna Sonnefeld, Geoffrey Bodenhausen, and Kirill Sheberstov[*]
Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure,
PSL University, Sorbonne Université, CNRS, 75005 Paris, France

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Long-lived states (LLS) involving pairs of magnetically inequivalent but chemically equivalent proton spins in aliphatic $(CH_2)_n$ chains can be excited by simultaneous application of weak selective radio frequency fields at n chemical shifts by polychromatic spin-lock induced crossing. The LLS are delocalized throughout the aliphatic chains by mixing of intrapair singlet states and by excitation of LLS comprising products of four and six spin operators. The measured lifetimes T_{LLS} in a model compound are about 5 times longer than T_1 and are strongly affected by interactions with macromolecules.

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The discovery of long-lived states (LLS) in nuclear magnetic resonance (NMR) has opened important perspectives [1–10]. If the lifetimes T_{LLS} are longer than the longitudinal relaxation times T_1, one can store nuclear spin hyperpolarization [11], observe hyperpolarized metabolites in magnetic resonance imaging [12], probe slow chemical exchange [13], determine slow diffusion rates [14,15], and detect weak interactions between potential drugs and target proteins [16–18]. Likewise, long-lived coherences can have lifetimes longer than T_2 or even T_1 [19–21].

In a pair of two spins A and A', the difference between the population of the singlet state $p(S_0^{AA'})$ and the mean population of the three triplet states $\langle p(T^{AA'})\rangle = \frac{1}{3}[p(T_{+1}^{AA'}) + p(T_0^{AA'}) + p(T_{-1}^{AA'})]$ is known as triplet-singlet *population imbalance*, which is immune against relaxation driven by intrapair dipole-dipole couplings [49]. Such a LLS can be described by a scalar product $\hat{\boldsymbol{I}}^A \cdot \hat{\boldsymbol{I}}^{A'}$, where $\hat{\boldsymbol{I}}^p = \hat{I}_x^p + \hat{I}_y^p + \hat{I}_z^p$, $p \in \{A, A'\}$. To excite such a LLS, the two spins should either have different resonance frequencies (chemical inequivalence) or have different interactions with other spins (magnetic inequivalence) [22,23]. An imbalance can also occur if the high spin temperature approximation is violated [24,25]. In systems with more than two spins, one can excite long-lived imbalances between states that belong to different symmetries of the spin permutation group [6,8,10,26–30].

In molecules that contain nearly equivalent spins, either with slightly different chemical shifts or with slightly different scalar couplings to neighboring spins, a two-spin LLS is almost an eigenstate and therefore does not require any radio frequency (rf) fields to be sustained [4,21,31]. Such molecules often require challenging chemical synthesis with isotopic enrichment of near-equivalent ^{13}C [6,7,31,32] or ^{15}N [10,33,34] spin pairs. Molecules that contain nearly equivalent proton spins have also been studied [4,35]. Here we show that LLS can be readily excited and observed in CH_2 groups of common aliphatic chains, which are ubiquitous in chemistry.

In achiral molecules that contain at least two neighboring CH_2 groups, the two protons of each CH_2 group are chemically equivalent (i.e., have the same chemical shifts), but are generally magnetically inequivalent (i.e., have different scalar couplings to neighboring CH_2 groups) [36]. Provided the substituents R and R' in a molecule $R(CH_2)_nR'$ are distinct, neighboring CH_2 subunits are often weakly coupled to each other in high magnetic fields; i.e., the out-of-pair J-couplings between them are much smaller than the difference between their chemical shifts. In a chain with $n = 3$ neighboring weakly coupled CH_2 groups, the spin system can be denoted by $AA'MM'XX'$, meaning that there are three pairs of chemically equivalent spins, that are however magnetically inequivalent [37]. The spin topology remains the same at arbitrary high fields. The relevant Hamiltonian and its (64×64)-dimensional matrix representation are discussed in the Supplemental Material [38].

So far, applications of spin-lock induced crossing (SLIC) [5,39] have been limited to monochromatic irradiation at a *single* radio frequency. Among other applications, SLIC has been used for heteronuclear systems of ^{13}C-enriched diphenylacetylene [6,40] and ^{15}N-enriched azobenzene [10,41], where the four *ortho* protons (ignoring the *meta* and *para* protons) and the two ^{15}N nuclei constitute an $AA'A''A'''XX'$ system. Somewhat counterintuitively, the application of a monochromatic SLIC sequence at the common shift ν_A of the four *ortho* protons excites a LLS that involves the two ^{15}N nuclei. This constitutes evidence for the delocalization of LLS along chains of coupled nuclei.

In this Letter, we introduce *polychromatic* SLIC (poly-SLIC). In $AA'MM'XX'$ systems, one may choose to apply one, two, or three carrier frequencies with weak rf amplitudes, yielding nine possible methods for magnetization-to-singlet conversion via double-quantum (DQ) or single-quantum (SQ) level anticrossings (LACs) as shown here.

1–3	Single-frequency SLIC with single-quantum LAC by irradiation at only one of the three shifts, ν_A, ν_M, or ν_X (single SLIC with SQ LAC).
4,5	Double-frequency SLIC with single-quantum LAC by simultaneous irradiation at the shifts ν_A and ν_M, or, equivalently, at the shifts ν_M and ν_X (double SLIC with SQ LAC of two neighboring CH_2 groups).
6	Double-frequency SLIC with single-quantum LAC by simultaneous irradiation at the shifts ν_A and ν_X of the two terminal CH_2 moieties (double SLIC with SQ LAC of two remote CH_2 groups).
7,8	Double-frequency SLIC with double-quantum LAC by simultaneous irradiation at two shifts ν_A and ν_M, or, equivalently, at the two shifts ν_M and ν_X (double SLIC with DQ LAC of two neighboring CH_2 groups).
9	Triple-frequency SLIC with DQ LAC by simultaneous irradiation at three shifts ν_A, ν_M, and ν_X.

The yields of these nine methods, along with a discussion of the SQ and DQ LACs, are given in the Supplemental Material [38]. As shown in Fig. 1, the magnetization is first converted into LLS by one of nine possible methods. The resulting LLS decays in the relaxation interval $\tau_{\rm rel}$. A T_{00} filter [42] then eliminates all off-diagonal elements of the density matrix, except for zero-quantum coherences. The remaining terms are then reconverted back into observable magnetization, again by one of nine possible methods (see Tables S2 and S3 in Supplemental Material [38]). The most efficient scheme uses triple SLIC excitation and single SLIC reconversion to maximize the observable magnetization of one of the three spin pairs.

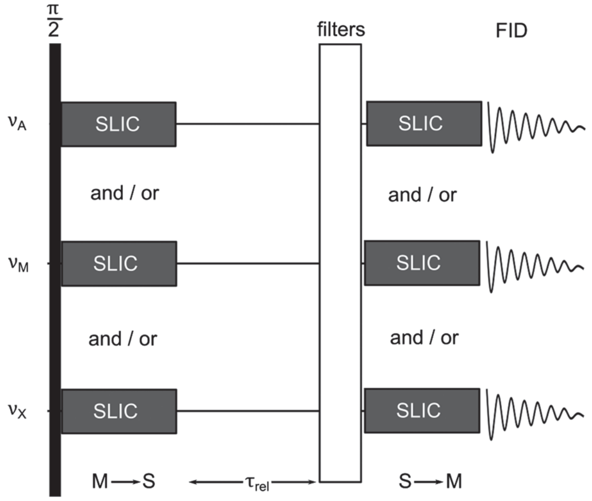

FIG. 1. Pulse sequences for poly-SLIC applied to an $AA'MM'XX'$ system of a chain of three neighboring CH_2 groups. All nine methods start by exciting transverse magnetization by a "hard" $(\pi/2)_x$ pulse, followed by the application of one, two, or three selective rf fields applied simultaneously at resonance frequencies (chemical shifts) ν_A and/or ν_M and/or ν_X with phases $\pm y$ [43]. The SLIC pulses convert the magnetization into LLS. The rf amplitudes must be twice the magnitude of the intrapair geminal J-coupling to achieve LLS excitation by single-quantum level anticrossing (SQ LAC), or equal to the magnitude of the intrapair J-coupling for double-quantum (DQ) LAC. Maximum efficiency is achieved when the pulse duration is the inverse of the magnitude of the difference between the two out-of-pair J-couplings for DQ LAC. For SQ LAC, maximum efficiency is achieved when the pulse duration is shorter by a factor of $\sqrt{2}$ (for the details see Supplemental Material). After a T_{00} filter [42], further SLIC pulses allow one to reconvert LLS into observable magnetization.

Numerical simulations have been performed to determine which LLS terms are excited by poly-SLIC (see Supplemental Material [38]). The following *delocalized* density operator terms can be obtained:

$$\begin{aligned}\hat{\sigma}_{\rm LLS} = {}& -\lambda_{AA'}N_2\hat{\mathbf{I}}^A\cdot\hat{\mathbf{I}}^{A'} - \lambda_{MM'}N_2\hat{\mathbf{I}}^M\cdot\hat{\mathbf{I}}^{M'} - \lambda_{XX'}N_2\hat{\mathbf{I}}^X\cdot\hat{\mathbf{I}}^{X'} - \lambda_{AA'MM'}N_4(\hat{\mathbf{I}}^A\cdot\hat{\mathbf{I}}^{A'})(\hat{\mathbf{I}}^M\cdot\hat{\mathbf{I}}^{M'}) - \lambda_{AA'XX'}N_4(\hat{\mathbf{I}}^A\cdot\hat{\mathbf{I}}^{A'})(\hat{\mathbf{I}}^X\cdot\hat{\mathbf{I}}^{X'})\\ & - \lambda_{MM'XX'}N_4(\hat{\mathbf{I}}^M\cdot\hat{\mathbf{I}}^{M'})(\hat{\mathbf{I}}^X\cdot\hat{\mathbf{I}}^{X'}) - \lambda_{AA'MM'XX'}N_6(\hat{\mathbf{I}}^A\cdot\hat{\mathbf{I}}^{A'})(\hat{\mathbf{I}}^M\cdot\hat{\mathbf{I}}^{M'})(\hat{\mathbf{I}}^X\cdot\hat{\mathbf{I}}^{X'}),\end{aligned} \tag{1}$$

with the norms $N_2 = (1/2\sqrt{3})$, $N_4 = 2/3$, and $N_6 = (8/3\sqrt{3})$. This equation gives a general form of the density matrix obtained after poly-SLIC, containing all long-lived terms found by numerical solution of the Liouville–von Neumann equation. The bilinear terms are familiar products of two spin operators, while the higher terms contain unusual products of four and six spin operators. Other terms relax rapidly or can be eliminated by filtration and phase cycling. The commutator between $1/3(\lambda_{AA'} + \lambda_{MM'} + \lambda_{XX'})N_2(\hat{\mathbf{I}}^A\cdot\hat{\mathbf{I}}^{A'} + \hat{\mathbf{I}}^M\cdot\hat{\mathbf{I}}^{M'} + \hat{\mathbf{I}}^X\cdot\hat{\mathbf{I}}^{X'})$ and the free-precession Hamiltonian yields a negligible residue. The same is true for the sum of the three four-spin terms, as well as for the six-spin term. Therefore, these terms are nearly invariant under free evolution, as evidenced by the weak oscillations during free evolution shown in Fig. 2.

One should distinguish two aspects of the delocalized nature of the LLS: (i) delocalization by mixing of spin states and (ii) delocalization by excitation of products comprising four- and six-spin states. A manifestation of *delocalization by mixing* is the experimental fact that a monochromatic SLIC pulse applied only to the AA' pair also excites LLS associated with the MM' and XX' pairs

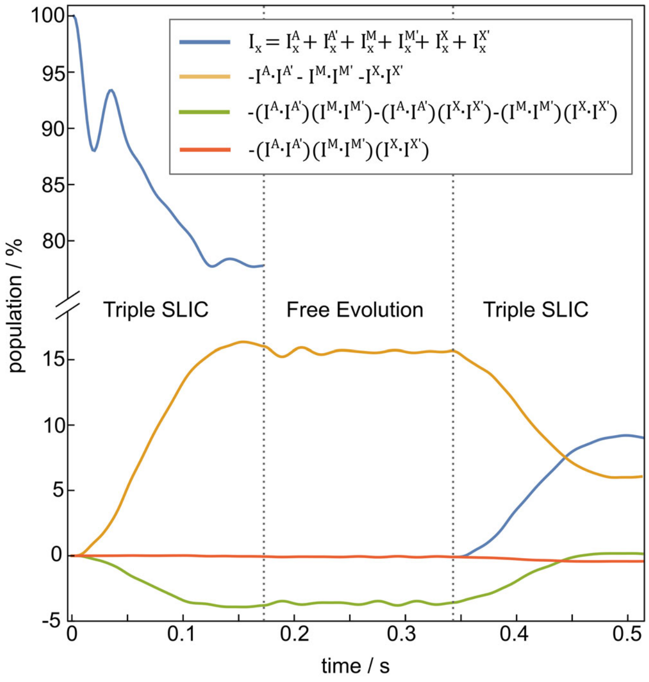

FIG. 2. Numerical simulations of the excitation and reconversion of three types of LLS in Eq. (1). The superposition of two-spin product terms (yellow) has coefficients above 15% after triple SLIC excitation, the four-spin product terms (green) have negative coefficients close to −4%, while the six-spin product term (red) has a negligible amplitude. The blue lines correspond to the trajectory of the total magnetization of the six spins that is partly converted into LLS and back. All simulations were carried out with *SpinDynamica* [44].

(Fig. 4). One can also create LLS that are delocalized over n neighboring CH_2 groups when the density operator is made up of a *product*, rather than a sum, of population imbalances, such as the six-spin term $(\hat{\mathbf{I}}^A \cdot \hat{\mathbf{I}}^{A'})(\hat{\mathbf{I}}^M \cdot \hat{\mathbf{I}}^{M'})(\hat{\mathbf{I}}^X \cdot \hat{\mathbf{I}}^{X'})$. Such states are rarely observed in NMR and may be of interest for quantum information processing.

Figure 3 shows a selection of experimental multiplets typical of the $AA'MM'XX'$ system of $NaSO_3CH_2CH_2CH_2Si(CH_3)_3$ (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt, also known as DSS), the methyl groups of which are commonly used as a chemical shift standard in aqueous solution. The multiplets obtained after conversion of magnetization into LLS and back are characteristic for a density operator of the form of Eq. (1). Their integrated intensities reach ca. 6% of the multiplet intensities in a conventional spectrum. Note the agreement between simulated and experimental multiplets. A simplification of multiplets such as those shown in Fig. 3 could be achieved by polychromatic homonuclear decoupling during the observation of the free induction decay [45], so that each multiplet would collapse to a single line.

Figure 4 shows decays of LLS as a function of the relaxation interval $\tau_{\rm rel}$. Four experiments have been performed with different SLIC excitation pulses but with the same reconversion SLIC applied at the ν_A frequency. The relaxation rates of LLS are dominated by out-of-pair dipole-dipole couplings, so that these rates are reduced by the ratio of the sixth power of the internuclear distances $(r_{AM}/r_{AA'})^6$ with respect to the longitudinal rates $1/T_1$ that are mostly determined by intrapair dipole-dipole couplings.

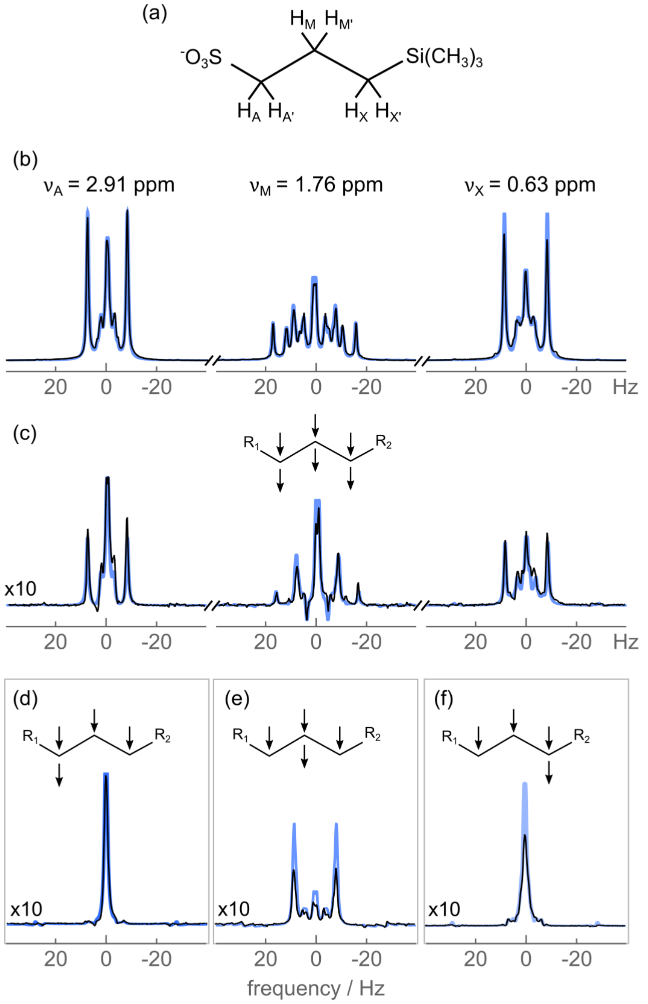

FIG. 3. (a) Molecular structure of $NaSO_3CH_2CH_2CH_2Si(CH_3)_3$ (DSS). (b) Multiplets of its $AA'MM'XX'$ proton system, excited by a “hard” $\pi/2$ pulse applied to the system in thermal equilibrium. Experimental multiplets (black) and simulated multiplets (blue) [46]. (c) Multiplets obtained by triple SLIC excitation of LLS followed, after a relaxation interval $\tau_{\rm rel} = 3$ s and a T_{00} filter, by reconversion into observable magnetization by triple SLIC irradiation. The arrows indicate at which chemical shifts the SLIC irradiation was applied. (d)–(f) Multiplets obtained in three separate experiments, each after triple SLIC excitation, evolution during $\tau_{\rm rel} = 3$ s, and a T_{00} filter. (d) Multiplet of the AA' pair obtained by single SLIC reconversion by irradiation at ν_A. (e) Multiplet of the MM' pair after irradiation at ν_M. (f) Multiplet of the XX' pair after irradiation at ν_X. Note the agreement between experimental and simulated spectra (black and blue lines, respectively). The vertical scales of the experimental multiplets in (c)–(f) were amplified by a factor 10. All spectra were obtained by addition of eight transients.

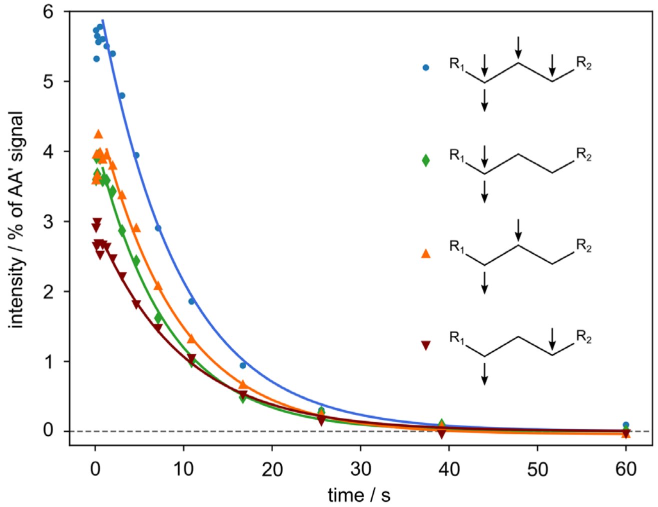

FIG. 4. Typical decays of long-lived states. The arrows indicate at which sites the SLIC irradiation is applied. Blue dots: LLS excitation by triple SLIC, reconversion by single SLIC at ν_A only ($T_{\mathrm{LLS}} = 9.3 \pm 0.4$ s). Green diamonds: LLS excitation and reconversion both by single SLIC at ν_A ($T_{\mathrm{LLS}} = 8.0 \pm 0.4$ s). Orange triangles: LLS excitation by single SLIC at ν_M and reconversion on a neighboring group by single SLIC at ν_A ($T_{\mathrm{LLS}} = 9.6 \pm 0.5$ s). Red inverted triangles: LLS excitation by single SLIC at ν_X and remote reconversion by single SLIC at ν_A ($T_{\mathrm{LLS}} = 9.7 \pm 0.4$ s). The solid lines correspond to monoexponential fits for $\tau_{\mathrm{rel}} > 0.84$ s, ignoring initial oscillations attributed to zero-quantum coherences. The largest amplitude is observed for triple SLIC excitation. The LLS lifetimes are at least 5 times longer than the corresponding longitudinal relaxation times $T_1^A \approx T_1^M \approx T_1^X \approx 1.5 \pm 0.03$ s.

Triple SLIC excitation gives amplitudes that are almost twice as large as single SLIC. The amplitude of the LLS signal excited by SLIC applied at the ν_X frequency gives the smallest amplitude, but it illustrates the delocalized character of the LLS, which results from the mixing of spin states. The experiment where both the excitation and the reconversion SLIC pulses are applied at one and the same frequency ν_A (green diamonds in Fig. 4) gives a T_{LLS} that is slightly shorter than the other experiments which yield similar T_{LLS}. This may be attributed to the fact that in this case the LLS is predominantly localized on the AA' pair (see Tables S1 and S2 in Supplemental Material [38]).

It is straightforward to excite LLS in potential drug molecules that contain two or more neighboring CH_2 groups. The contrast between the relaxation rates $R_{\mathrm{LLS}} = 1/T_{\mathrm{LLS}}$ of free and partly bound drug molecules allows one to determine their affinity (binding constants) for macromolecules such as proteins [16–18]. Binding may partially lift the chemical equivalence, i.e., convert an $AA'MM'XX'$ system into an $ABMNXY$ system. This will contribute to a leakage between intrapair T_0 and S_0 states, thus leveling out the population imbalances, reducing T_{LLS}, and enhancing the contrast between free and bound drug molecules. By way of example, 5 mM DSS has been titrated with bovine serum albumin (BSA) over the range $0.5 \leq [\mathrm{BSA}] \leq 20\ \mu\mathrm{M}$. As shown in the Supplemental Material, the lifetime of the LLS of DSS drops steeply by almost a factor of 10 upon addition of BSA at very low concentrations $[\mathrm{BSA}]/[\mathrm{DSS}] > 10^{-4}$, whereas the proton chemical shifts, T_1 and T_2 are barely affected for $[\mathrm{BSA}] < 20\ \mu\mathrm{M}$. The contrast may be amplified by shuttling the sample to a lower field during the relaxation interval τ_{rel} [47].

The poly-SLIC method opens several prospects for future work. In a chain with n CH_2 groups, various delocalized states can be addressed via different entrance ports, and can be read out at the same or other output ports. Similar LLS can be found in many chemical compounds, e.g., in neurotransmitters like gamma-aminobutyric acid (GABA) [48].

To conclude, delocalized long-lived states encompassing all six proton spins in $AA'MM'XX'$ systems in chains of three neighboring aliphatic CH_2 groups have been excited and observed by poly-SLIC. The lifetimes T_{LLS} of the long-lived states were about 5 times longer than T_1 and were dramatically shortened when molecules that carry long-lived states interacted with a protein. This could be useful for screening of drug molecules without the need for labeling with fluorescent tags or other chemical modifications.

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[*] Corresponding author.
kirill.sheberstov@ens.psl.eu

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[38] See Supplemental Material at http://link.aps.org/supplemental/10.1103/PhysRevLett.129.183203 for the theory of level anti-crossings under polychromatic SLIC pulses in $AA'MM'XX'$ systems; calculation of the yields of excitation and reconversion of LLS; scalar couplings in DSS; titration with bovine serum albumin; experimental details.

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