



HAL
open science

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

Anna Sonnefeld, Geoffrey Bodenhausen, Kirill Sheberstov

► **To cite this version:**

Anna Sonnefeld, Geoffrey Bodenhausen, Kirill Sheberstov. Polychromatic Excitation of Delocalized Long-Lived Proton Spin States in Aliphatic Chains. *Physical Review Letters*, 2022, 129 (18), pp.183203. 10.1103/PhysRevLett.129.183203 . hal-03989530

HAL Id: hal-03989530

<https://hal.science/hal-03989530>

Submitted on 14 Feb 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Anna Sonnefeld, Geoffrey Bodenhausen, Kirill Sheberstov*

Laboratoire des biomolécules, LBM, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France

23st September 2022

*Corresponding author

kirill.sheberstov@ens.psl.eu

Abstract

Long-lived states (LLS) involving pairs of magnetically inequivalent but chemically equivalent proton spins in aliphatic $(\text{CH}_2)_n$ chains can be excited by simultaneous application of weak selective radio-frequency (RF) fields at n chemical shifts by polychromatic spin-lock induced crossing (poly-SLIC). The LLS are delocalized throughout the aliphatic chains by mixing of intrapair singlet states and by excitation of LLS comprising products of four or 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.

Key words

Long-lived states, Spin-lock induced crossing, Level anti crossings, Drug screening, Chains of addressable qubits, Quantum information processing

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 (MRI) [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 (LLCs) 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 intra-pair dipole-dipole couplings [18]. Such an LLS can be described by a scalar product $\hat{I}^A \cdot \hat{I}^{A'}$, where $\hat{I}^p = \hat{I}_x^p + \hat{I}_y^p + \hat{I}_z^p$, $p \in \{A, A'\}$. To excite such an LLS, the two spins should have either different resonance frequencies (chemical inequivalence), or have different interactions with other spins (magnetic inequivalence) [24,25]. An imbalance can also occur if the high spin temperature approximation is violated [26,27]. 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 [6,8,10,28–32].

In molecules that contain nearly equivalent spins, either with slightly different chemical shifts, or with slightly different scalar couplings to neighbouring spins, a two-spin LLS is almost an eigenstate and therefore does not require any radio-frequency (RF) fields to be sustained [4,21,33]. Such molecules often require challenging chemical synthesis with isotopic enrichment of near-equivalent ^{13}C [6,7,33,34] or ^{15}N [10,35,36] spin pairs. Molecules that contain nearly equivalent proton spins have also been studied [4,37]. 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 neighbouring CH_2 groups, the two protons of each CH_2 group are chemically equivalent (i.e., have the same chemical shifts) but they are generally magnetically inequivalent (i.e., have different scalar couplings to neighbouring CH_2

groups) [38]. Provided the substituents R and R' in a molecule $R(\text{CH}_2)_n\text{R}'$ 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 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 [39]. The spin topology remains the same at arbitrary high fields. The relevant Hamiltonian and its 64×64 - dimensional matrix representation are discussed in Supplemental Information.

So far, applications of spin-lock induced crossing (SLIC) [5,40] 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 [6,41] and ^{15}N -enriched azobenzene [10,42], 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 counter-intuitively, the application of a monochromatic SLIC sequence at the common shift ν_A of the four *ortho* protons excites an LLS that involves the two ^{15}N nuclei. This constitutes evidence for the delocalization of LLS along chains of coupled nuclei.

In this work, 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 9 possible methods for magnetization-to-singlet conversion via double-quantum (DQ) or single-quantum (SQ) level anti-crossings (LACs):

- 1-3 Single-frequency SLIC with single-quantum LAC by irradiation at only one of the three shifts ν_A or 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 neighbouring 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 neighbouring CH_2 groups)
- 9 Triple-frequency SLIC with DQ LAC by simultaneous irradiation at three shifts ν_A , ν_M , and ν_X .

The efficiencies (quantum yields) of these 9 methods, along with a discussion of the SQ and DQ LACs, are given in the Supplemental Material. As shown in Figure 1, the magnetization is first converted into LLS by one of 9 possible methods. The resulting LLS decays in the relaxation interval τ_{rel} . A T_{00} filter [27] then eliminates all off-diagonal elements of the density matrix, except for some zero-quantum coherences. The remaining terms are then re-converted back into observable magnetization, again by one of 9 possible methods (see Tables S2 and S3). The most efficient scheme uses triple SLIC excitation and single SLIC reconversion to maximize the observable magnetization of one of the three spin pairs.

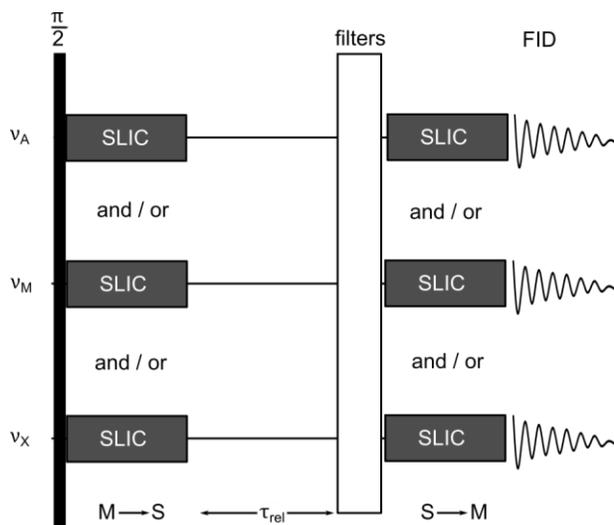


Figure 1. Pulse sequences for poly-SLIC applied to an $AA'MM'XX'$ system of a chain of 3 neighboring CH_2 groups. All 9 methods start by exciting transverse magnetization by a 'hard' $(\pi/2)_x$ pulse, followed by the simultaneous application of one, two or three selective RF fields applied simultaneously at resonance frequencies (chemical shifts) ν_A and/or ν_M and/or ν_X with phases $\pm y$. The SLIC pulses convert the magnetization into LLS. The RF amplitudes must be $\nu_{RF} = 2J_{intra}$ to achieve LLS excitation by single-quantum level anti-crossing (SQ LAC), or $\nu_{RF} = J_{intra}$ for double-quantum (DQ) LAC. Maximum efficiency is achieved when $\tau_{SLIC}^{SQ} = 1/(|\sqrt{2}\Delta J|)$ for SQ LAC and $\tau_{SLIC}^{DQ} = \sqrt{2}\tau_{SLIC}^{SQ}$ for DQ LAC. After a T_{00} filter [43], further SLIC sequences allow one to reconvert LLS into observable magnetization.

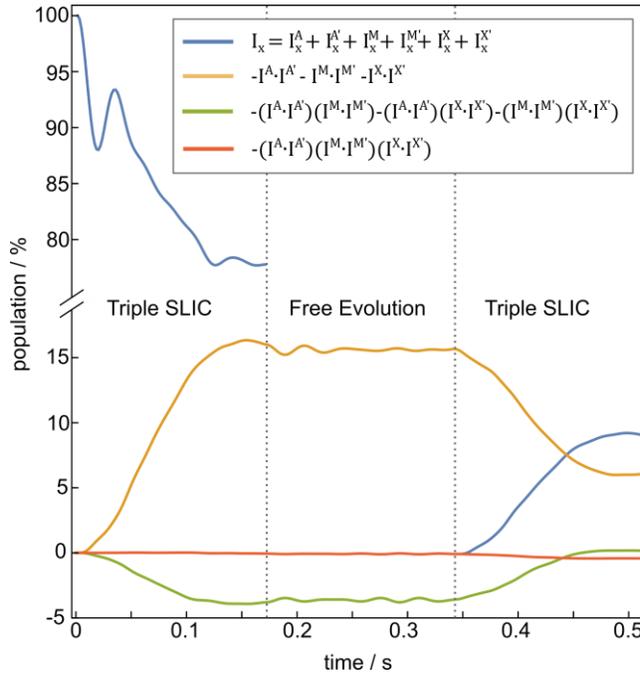


Figure 2. Numerical simulations of the excitation and reconversion of three types of delocalized long-lived states (LLS) in Eq. (1). The superposition of two-spin product terms (yellow) has coefficients above 15% after triple SLIC excitation, the four-spin product terms (green) have negative coefficients 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 6 spins that is partly converted into LLS and back. All simulations were carried out with Spin Dynamica [44].

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

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

With the norms $N_2 = \frac{1}{2\sqrt{3}}$, $N_4 = \frac{2}{3}$, $N_6 = \frac{8}{3\sqrt{3}}$. 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 2 spin operators, while the higher terms contain unusual products of 4 and 6 spin operators. Other terms relax rapidly or can be eliminated by filtration and phase cycling. The commutator between $\frac{1}{3} (\lambda_{AA'} + \lambda_{MM'} + \lambda_{XX'}) N_2 (\hat{\mathbf{I}}^A \cdot \hat{\mathbf{I}}^{A'} + \hat{\mathbf{I}}^M \cdot \hat{\mathbf{I}}^{M'} + \hat{\mathbf{I}}^X \cdot \hat{\mathbf{I}}^{X'})$ 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 Figure 2.

One should distinguish two aspects of the delocalized nature of the LLS: (i) delocalization by mixing of two- and four-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 (Figure 4). One can also create LLS that are delocalized over n neighboring CH_2 groups because 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 $\text{NaSO}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{Si}(\text{CH}_3)_3$ (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 the $\hat{\sigma}_{LLS}$ density operator (Eq. 1). Their integrated intensities reach *ca.* 6% of a conventional spectrum. Note the agreement between simulated and experimental multiplets. A dramatic simplification of multiplets such as those shown in Figure 3 could be achieved by polychromatic homonuclear decoupling during the observation of the FID [45], so that each multiplet would collapse to a single line.

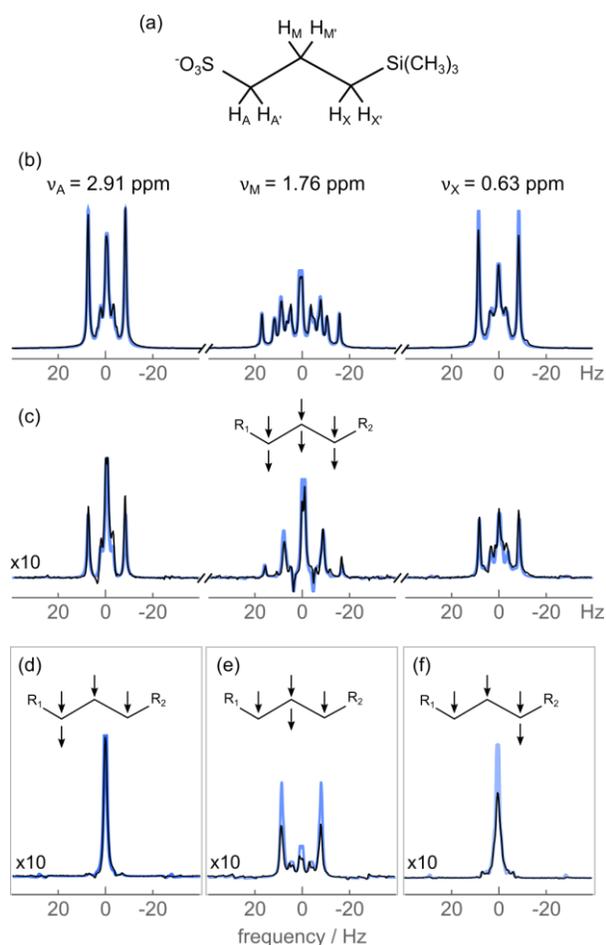


Figure 3. (a) Molecular structure of $\text{NaSO}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{Si}(\text{CH}_3)_3$ (DSS). (b) Multiplets of its $AA'MM'XX'$ proton system, excited by a 'hard' $\pi/2$ pulse applied to thermal equilibrium. (Black) Experimental multiplets and (blue) simulated multiplets [46] (c) Multiplets obtained by triple SLIC excitation of LLS followed, after a relaxation interval $\tau_{rel} = 3$ 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 is applied. (d-f) Multiplets obtained in three separate experiments, each after triple SLIC excitation, evolution during $\tau_{rel} = 3$ 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 intensities of the experimental multiplets in (c)-(f) were scaled by factor 10. All the spectra were obtained by addition of 8 transients.

Figure 4 shows decays of LLS as a function of the relaxation interval τ_{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 6th power of the internuclear distances $(r_{AM}/r_{AA'})^6$ with respect to the longitudinal rates $1/T_1$ that are mostly determined by intra-pair dipole-dipole couplings. 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 Figure 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, S2).

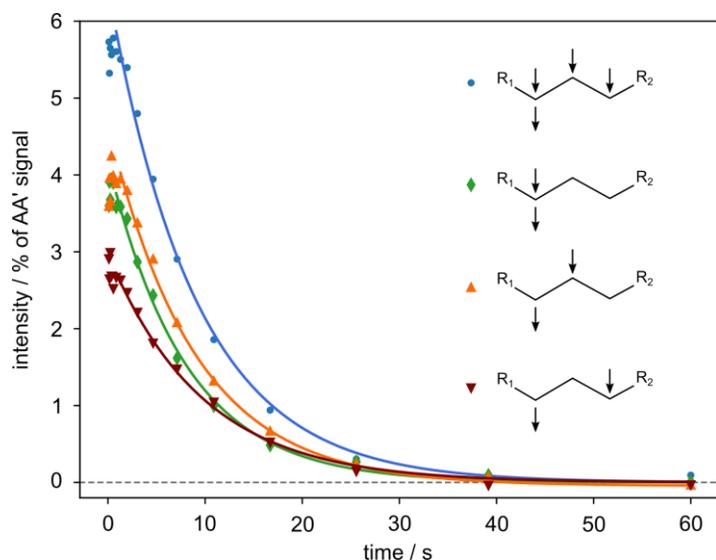


Figure 4. Typical decays of long-lived states. The arrows indicate at which sites the SLIC irradiation is applied. (Blue dots) LLS excitation by triple SLIC, reconversion by single SLIC at ν_A only ($T_{LLS} = 9.3 \pm 0.4$ s). (Green diamonds) LLS excitation and reconversion both by single SLIC at ν_A ($T_{LLS} = 8.0 \pm 0.4$ s). (Orange triangles) LLS excitation by single SLIC at ν_M and reconversion on a neighboring group by single SLIC at ν_A ($T_{LLS} = 9.6 \pm 0.5$ s). (Red downward pointing triangles) LLS excitation by single SLIC at ν_X and remote reconversion by single SLIC at ν_A ($T_{LLS} = 9.7 \pm 0.4$ s). The solid lines correspond to mono-exponential fits for $\tau_{rel} > 0.84$ 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.

It is straightforward to excite LLS in potential drug molecules that contain two or more neighboring CH₂ groups. The contrast between the relaxation rates $R_{LLS} = 1/T_{LLS}$ of free and partly bound drug molecules allows one to determine the affinity (binding constants) for macromolecules such as proteins [16–18]. Binding may partly lift the chemical equivalence, i.e., convert an $AA'MM'XX'$ system into an $ABMNXY$ system. This will contribute to a leakage between intra-pair T_0 and S_0 states, thus levelling 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 $0.5 \mu\text{M} \leq [\text{BSA}] \leq 20 \mu\text{M}$. As shown in the Supplemental Material, the lifetime of the LLS of DSS drops steeply by almost factor of 10 upon addition of BSA at very low concentrations $[\text{BSA}]/[\text{DSS}] > 10^{-4}$, whereas the

proton chemical shifts, T_1 and T_2 are barely affected for $[BSA] < 20 \mu\text{M}$. The contrast could 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 delocalised 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 [48], e.g., in neurotransmitters like gamma-aminobutyric acid (GABA).

To conclude, delocalized long-lived states encompassing all 6 proton spins in $AA'MM'XX'$ systems in chains of 3 neighbouring aliphatic CH_2 groups have been excited and observed by polychromatic spin-lock induced crossing (poly-SLIC). The lifetimes T_{LLS} of the long-lived states are typically 5 times longer than T_1 . The lifetimes T_{LLS} are dramatically shortened when molecules that carry long-lived states interact with macromolecules such as proteins. This could be useful for screening of drug molecules without the need for labelling with fluorescent tags or other chemical modifications.

Acknowledgements

We are indebted to Dr Philippe Pelupessy for stimulating discussions, to the CNRS and the ENS for support, and to the European Research Council (ERC) for the Synergy grant “Highly Informative Drug Screening by Overcoming NMR Restrictions” (HISCORE, grant agreement number 951459).

References

- [1] M. Carravetta, O. G. Johannessen, and M. H. Levitt, *Beyond the T_1 Limit: Singlet Nuclear Spin States in Low Magnetic Fields*, Phys. Rev. Lett. **92**, 153003 (2004).
- [2] M. Carravetta and M. H. Levitt, *Long-Lived Nuclear Spin States in High-Field Solution NMR*, J. Am. Chem. Soc. **126**, 6228 (2004).
- [3] R. Sarkar, P. R. Vasos, and G. Bodenhausen, *Singlet-State Exchange NMR Spectroscopy for the Study of Very Slow Dynamic Processes*, J. Am. Chem. Soc. **129**, 328 (2007).
- [4] M. C. D. Tayler and M. H. Levitt, *Singlet Nuclear Magnetic Resonance of Nearly-Equivalent Spins*, Phys. Chem. Chem. Phys. **13**, 5556 (2011).
- [5] S. J. DeVience, R. L. Walsworth, and M. S. Rosen, *Preparation of Nuclear Spin Singlet States Using Spin-Lock Induced Crossing*, Phys. Rev. Lett. **111**, 173002 (2013).
- [6] Y. Feng, T. Theis, X. Liang, Q. Wang, P. Zhou, and W. S. Warren, *Storage of Hydrogen Spin Polarization in Long-Lived $^{13}\text{C}_2$ Singlet Order and Implications for Hyperpolarized Magnetic Resonance Imaging*, J. Am. Chem. Soc. **135**, 9632 (2013).

- [7] K. Claytor, T. Theis, Y. Feng, J. Yu, D. Gooden, and W. S. Warren, *Accessing Long-Lived Disconnected Spin-1/2 Eigenstates through Spins $> 1/2$* , *J. Am. Chem. Soc.* **136**, 15118 (2014).
- [8] G. Stevanato, S. S. Roy, J. Hill-Cousins, I. Kuprov, L. J. Brown, R. C. D. Brown, G. Pileio, and M. H. Levitt, *Long-Lived Nuclear Spin States Far from Magnetic Equivalence*, *Phys. Chem. Chem. Phys.* **17**, 5913 (2015).
- [9] A. N. Pravdivtsev, A. S. Kiryutin, A. V. Yurkovskaya, H.-M. Vieth, and K. L. Ivanov, *Robust Conversion of Singlet Spin Order in Coupled Spin-1/2 Pairs by Adiabatically Ramped RF-Fields*, *J. Magn. Reson.* **273**, 56 (2016).
- [10] K. F. Sheberstov, H.-M. Vieth, H. Zimmermann, B. A. Rodin, K. L. Ivanov, A. S. Kiryutin, and A. V. Yurkovskaya, *Generating and Sustaining Long-Lived Spin States in 15 N, 15 N'-Azobenzene*, *Sci. Rep.* **9**, 1 (2019).
- [11] P. R. Vasos, A. Comment, R. Sarkar, P. Ahuja, S. Jannin, J.-P. Ansermet, J. A. Konter, P. Hautle, B. van den Brandt, and G. Bodenhausen, *Long-Lived States to Sustain Hyperpolarized Magnetization*, *PNAS* **106**, 18469 (2009).
- [12] D. B. Burueva, J. Eills, J. W. Blanchard, A. Garcon, R. Picazo-Frutos, K. V. Kovtunov, I. V. Koptug, and D. Budker, *Chemical Reaction Monitoring Using Zero-Field Nuclear Magnetic Resonance Enables Study of Heterogeneous Samples in Metal Containers*, *Angew. Chem. Int. Ed. Engl.* **59**, 17026 (2020).
- [13] C. Bengs, L. Dagys, G. A. I. Moustafa, J. W. Whipham, M. Sabba, A. S. Kiryutin, K. L. Ivanov, and M. H. Levitt, *Nuclear Singlet Relaxation by Chemical Exchange*, *J. Chem. Phys.* **155**, 124311 (2021).
- [14] S. Cavadini, J. Dittmer, S. Antonijevic, and G. Bodenhausen, *Slow Diffusion by Singlet State NMR Spectroscopy*, *J. Am. Chem. Soc.* **127**, 15744 (2005).
- [15] M. C. Tourell, I.-A. Pop, L. J. Brown, R. C. D. Brown, and G. Pileio, *Singlet-Assisted Diffusion-NMR (SAD-NMR): Redefining the Limits When Measuring Tortuosity in Porous Media*, *Phys. Chem. Chem. Phys.* **20**, 13705 (2018).
- [16] N. Salvi, R. Buratto, A. Borner, S. Ulzega, I. Rentero Rebollo, A. Angelini, C. Heinis, and G. Bodenhausen, *Boosting the Sensitivity of Ligand-Protein Screening by NMR of Long-Lived States*, *J. Am. Chem. Soc.* **134**, 11076 (2012).
- [17] R. Buratto, D. Mammoli, E. Chiarparin, G. Williams, and G. Bodenhausen, *Exploring Weak Ligand-Protein Interactions by Long-Lived NMR States: Improved Contrast in Fragment-Based Drug Screening*, *Angew. Chem. Int. Ed.* **53**, 11376 (2014).
- [18] R. Buratto, D. Mammoli, E. Canet, and G. Bodenhausen, *Ligand-Protein Affinity Studies Using Long-Lived States of Fluorine-19 Nuclei*, *J. Med. Chem.* **59**, 1960 (2016).
- [19] G. Pileio, M. Carravetta, and M. H. Levitt, *Extremely Low-Frequency Spectroscopy in Low-Field Nuclear Magnetic Resonance*, *Phys. Rev. Lett.* **103**, 083002 (2009).
- [20] R. Sarkar, P. Ahuja, P. R. Vasos, and G. Bodenhausen, *Long-Lived Coherences for Homogeneous Line Narrowing in Spectroscopy*, *Phys. Rev. Lett.* **104**, 053001 (2010).
- [21] K. F. Sheberstov, A. S. Kiryutin, C. Bengs, J. T. Hill-Cousins, L. J. Brown, R. C. D. Brown, G. Pileio, M. H. Levitt, A. V. Yurkovskaya, and K. L. Ivanov, *Excitation of Singlet-Triplet Coherences in Pairs of Nearly-Equivalent Spins*, *Phys. Chem. Chem. Phys.* **21**, 6087 (2019).
- [22] B. A. Rodin, C. Bengs, A. S. Kiryutin, K. F. Sheberstov, L. J. Brown, R. C. D. Brown, A. V. Yurkovskaya, K. L. Ivanov, and M. H. Levitt, *Algorithmic Cooling of Nuclear Spins Using Long-Lived Singlet Order*, *J. Chem. Phys.* **152**, 164201 (2020).
- [23] G. Pileio, *Relaxation Theory of Nuclear Singlet States in Two Spin-1/2 Systems*, *Prog. Nucl. Magn. Reson. Spectrosc.* **56**, 217 (2010).
- [24] G. Pileio and M. H. Levitt, *J-Stabilization of Singlet States in the Solution NMR of Multiple-Spin Systems*, *J. Magn. Reson.* **187**, 141 (2007).

- [25] M. B. Franzoni, L. Buljubasich, H. W. Spiess, and K. Münnemann, *Long-Lived 1H Singlet Spin States Originating from Para-Hydrogen in Cs-Symmetric Molecules Stored for Minutes in High Magnetic Fields*, *J. Am. Chem. Soc.* **134**, 10393 (2012).
- [26] M. C. D. Tayler, I. Marco-Rius, M. I. Kettunen, K. M. Brindle, M. H. Levitt, and G. Pileio, *Direct Enhancement of Nuclear Singlet Order by Dynamic Nuclear Polarization*, *J. Am. Chem. Soc.* **134**, 7668 (2012).
- [27] D. Mammoli, N. Salvi, J. Milani, R. Buratto, A. Bornet, A. A. Sehgal, E. Canet, P. Pelupessy, D. Carnevale, S. Jannin, G. Bodenhausen, *Challenges in Preparing, Preserving and Detecting Para -Water in Bulk: Overcoming Proton Exchange and Other Hurdles*, *Phys. Chem. Chem. Phys.* **17**, 26819 (2015).
- [28] G. Pileio, M. Concistrè, M. Carravetta, and M. H. Levitt, *Long-Lived Nuclear Spin States in the Solution NMR of Four-Spin Systems*, *J. Magn. Reson.* **182**, 353 (2006).
- [29] E. Vinogradov and A. K. Grant, *Hyperpolarized Long-Lived States in Solution NMR: Three-Spin Case Study in Low Field*, *J. Magn. Reson.* **194**, 46 (2008).
- [30] A. K. Grant and E. Vinogradov, *Long-Lived States in Solution NMR: Theoretical Examples in Three- and Four-Spin Systems*, *J. Magn. Reson.* **193**, 177 (2008).
- [31] P. Ahuja, R. Sarkar, P. R. Vasos, and G. Bodenhausen, *Long-Lived States in Multiple-Spin Systems*, *ChemPhysChem* **10**, 2217 (2009).
- [32] H. J. Hogben, P. J. Hore, and I. Kuprov, *Multiple Decoherence-Free States in Multi-Spin Systems*, *J. Magn. Reson.* **211**, 217 (2011).
- [33] G. Pileio, J. T. Hill-Cousins, S. Mitchell, I. Kuprov, L. J. Brown, R. C. D. Brown, and M. H. Levitt, *Long-Lived Nuclear Singlet Order in Near-Equivalent 13C Spin Pairs*, *J. Am. Chem. Soc.* **134**, 17494 (2012).
- [34] G. Stevanato, J. T. Hill-Cousins, P. Håkansson, S. S. Roy, L. J. Brown, R. C. D. Brown, G. Pileio, and M. H. Levitt, *A Nuclear Singlet Lifetime of More than One Hour in Room-Temperature Solution*, *Angew. Chem. Int. Ed. Engl.* **54**, 3740 (2015).
- [35] S. J. Elliott, P. Kadeřávek, L. J. Brown, M. Sabba, S. Glöggler, D. J. O'Leary, R. C. D. Brown, F. Ferrage, and M. H. Levitt, *Field-Cycling Long-Lived-State NMR of 15N2 Spin Pairs*, *Mol. Phys.* **117**, 861 (2019).
- [36] K. Shen, A. W. J. Logan, J. F. P. Colell, J. Bae, G. X. Ortiz, T. Theis, W. S. Warren, S. J. Malcolmson, and Q. Wang, *Diazirines as Potential Molecular Imaging Tags: Probing the Requirements for Efficient and Long-Lived SABRE-Induced Hyperpolarization*, *Angew. Chem. Int. Ed. Engl.* **56**, 12112 (2017).
- [37] J. Eills, G. Stevanato, C. Bengs, S. Glöggler, S. J. Elliott, J. Alonso-Valdesueiro, G. Pileio, and M. H. Levitt, *Singlet Order Conversion and Parahydrogen-Induced Hyperpolarization of 13C Nuclei in near-Equivalent Spin Systems*, *J. Magn. Reson.* **274**, 163 (2017).
- [38] G. Schumpf, *NMR Spectra of Monosubstituted Alkanes I. N-Propyl Derivatives*, *J. Magn. Reson.* (1969) **6**, 243 (1972).
- [39] J. A. Pople, W. G. Schneider, and H. J. Bernstein, *The Analysis of Nuclear Magnetic Resonance Spectra: II. Two Pairs of Two Equivalent Nuclei*, *Can. J. Chem.* **35**, 1060 (1957).
- [40] S. J. DeVience, M. Greer, S. Mandal, and M. S. Rosen, *Homocoupling J-Coupling Spectroscopy at Low Magnetic Fields Using Spin-Lock Induced Crossing***, *ChemPhysChem* **22**, 2128 (2021).
- [41] Y. Feng, T. Theis, T.-L. Wu, K. Claytor, and W. S. Warren, *Long-Lived Polarization Protected by Symmetry*, *J. Chem. Phys.* **141**, 134307 (2014).
- [42] K. F. Sheberstov, H.-M. Vieth, H. Zimmermann, K. L. Ivanov, A. S. Kiryutin, and A. V. Yurkovskaya, *Cis Versus Trans-Azobenzene: Precise Determination of NMR Parameters and Analysis of Long-Lived States of 15N Spin Pairs*, *Appl. Magn. Reson.* **49**, 293 (2018).
- [43] M. C. D. Tayler, *Chapter 10: Filters for Long-Lived Spin Order*, in *Long-Lived Nuclear Spin Order* (The Royal Society of Chemistry, 2020), pp. 188–208.
- [44] C. Bengs and M. H. Levitt, *SpinDynamica: Symbolic and Numerical Magnetic Resonance in a Mathematica Environment*, *Magn. Reson. Chem.* **56**, 374 (2018).

- [45] D. Carnevale, T. F. Segawa, and G. Bodenhausen, *Polychromatic Decoupling of a Manifold of Homonuclear Scalar Interactions in Solution-State NMR*, *Eur. J. Chem.* **18**, 11573 (2012).
- [46] D. A. Cheshkov, K. F. Sheberstov, D. O. Sinitsyn, and V. A. Chertkov, *ANATOLIA: NMR software for spectral analysis of total lineshape*, *Magn. Reson. Chem.* **56**, 449 (2018).
- [47] Z. Wang, S. Pisano, V. Ghini, P. Kadeřávek, M. Zachrdla, P. Pelupessy, M. Kazmierczak, T. Marquardsen, J.-M. Tyburn, G. Bouvignies, G. Parigi, C. Luchinat, F. Ferrage, *Detection of Metabolite–Protein Interactions in Complex Biological Samples by High-Resolution Relaxometry: Toward Interactomics by NMR*, *J. Am. Chem. Soc.* **143**, 9393 (2021).
- [48] A. Sonnefeld, A. Razanahoera, G. Bodenhausen, and K. Sheberstov, Long-Lived States of Methylene Protons in Achiral Molecules, arXiv:2207.08608.