



Long-Lived States Involving a Manifold of Fluorine-19 Spins in Fluorinated Aliphatic Chains

Coline Wiame, Sebastiaan Van Dyck, Kirill Sheberstov, Aiky Razanahoera and Geoffrey Bodenhausen

Chimie Physique et Chimie du Vivant (CPCV, UMR 8228), Département de Chimie, École Normale Supérieure, PSL
5 University, Sorbonne Université, CNRS, 75005 Paris, France

Correspondence to: Kirill Sheberstov (kirill.sheberstov@ens.psl.eu)

Abstract. Long-lived states (LLS) have lifetimes T_{LLS} that exceed longitudinal spin-lattice relaxation times T_1 . In this study, lifetimes $T_{\text{LLS}}(^{19}\text{F})$ have been measured in three different achiral per- and polyfluoroalkyl substances (PFAS) containing 2 or 3 consecutive CF_2 groups. In a static magnetic field $B_0 = 11.6$ T, the lifetimes $T_{\text{LLS}}(^{19}\text{F})$ exceed the longitudinal relaxation
10 times $T_1(^{19}\text{F})$ by about a factor 3. The lifetimes $T_{\text{LLS}}(^{19}\text{F})$ can be strongly affected by binding to macromolecules, a feature that can be exploited for screening of fluorinated drugs. Both $T_{\text{LLS}}(^{19}\text{F})$ and $T_1(^{19}\text{F})$ should be longer at low fields where relaxation due to the chemical shift anisotropy (CSA) of ^{19}F is less effective, as will be shown elsewhere.

1 Introduction

Many applications of long-lived states (LLS) have been illustrated for pairs of ^{13}C nuclei, for pairs of ^1H , and more rarely, for
15 pairs of ^{19}F nuclei. The slow decays of LLS have been exploited for the determination of small diffusion coefficients (Cavadini et al., 2005), for measurements of slow chemical exchange (Sarkar et al., 2007), for the storage of hyperpolarization (Vasos et al., 2009; Pileio et al., 2012; Kiryutin et al., 2019), for investigations of weak ligand-protein binding (Salvi et al., 2012), and for studies of tortuosity in porous media (Dumez et al., 2014; Pileio et al., 2015; Pileio and Ostrowska, 2017; Tourell et al., 2018; Melchiorre et al., 2023).

20 1.1 Fluorinated Drugs

Many drugs contain one or more fluorine atoms since pharmacokinetic studies have shown that their lifetimes *in vivo* are favored because C-F bonds are harder to break down by enzymes than C-H bonds (Shah and Westwell, 2007). Fluorinated drugs also have the advantage of being easy to track by NMR and MRI, since the sensitivity of ^{19}F NMR is comparable to that of ^1H NMR, while the absence of background signals offers decisive advantages (Buchholz and Pomerantz, 2021). For drug
25 screening, it is important to achieve the best possible contrast between the response of a ligand L that binds to a target protein P and a molecule that fails to do so. For ligands that bind to a target protein, good contrast can be achieved in ^{19}F NMR through the combined effects of binding on the chemical shifts and on the correlation times of rotational diffusion. The latter effect leads to line-broadening by homogeneous transverse T_2 relaxation. In addition, chemical exchange in the intermediate regime



can also contribute to line-broadening (Buchholz and Pomerantz, 2021). The latter two effects can be distinguished by comparison of, on the one hand, conventional Carr-Purcell-Meiboom-Gill (CPMG) echo trains with high repetition rates required to suppress echo modulations due to homonuclear scalar couplings $^nJ(^{19}\text{F}, ^{19}\text{F})$, which also inhibit echo decays due to intermediate exchange, and, on the other hand, slow CPMG echo trains using the so-called “perfect echoes” to eliminate the effects of $^nJ(^{19}\text{F}, ^{19}\text{F})$ couplings while retaining the effects of intermediate exchange (Takegoshi et al., 1989; Aguilar et al., 2012; Lorz et al., 2025). An LLS involving at least two ^{19}F spins can also provide good contrast (Buratto et al., 2016).

1.2 Long-Lived States

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 (Sonnefeld et al., 2022a). Such population imbalances are also known as LLSs since their lifetimes T_{LLS} can greatly exceed T_1 and T_2 . Such states can be described by scalar products of the type $\hat{I}^A \cdot \hat{I}^{A'}$, where $\hat{I}^p = (\hat{I}_x^p, \hat{I}_y^p, \hat{I}_z^p)$, $p \in \{A, A'\}$. In an achiral aliphatic chain with 6 spins, described by AA'MM'XX' in Pople's notation, one can excite not only two-spin order terms $\hat{I}^A \cdot \hat{I}^{A'}$ and/or $\hat{I}^M \cdot \hat{I}^{M'}$ and/or $\hat{I}^X \cdot \hat{I}^{X'}$ but also 4-spin order terms such as $\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^M \cdot \hat{I}^{M'}$ and/or $\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$ and/or $\hat{I}^M \cdot \hat{I}^{M'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$. One can also, in principle, excite a 6-spin order term $\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^M \cdot \hat{I}^{M'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$, although simulations for protons in aliphatic chains indicate a low yield of the excitation of such a 6-spin term. It is challenging to determine separately the coefficients of all seven possible products in a 6-spin system since one usually creates admixtures. Since experiments indicate that their decay constants are often very similar, admixtures of different products tend to relax with mono-exponential decays. These remarks apply to many molecules containing either ^{19}F or ^1H nuclei.

In some molecules, two ^{19}F spins have different chemical shifts, e.g., in many di-fluoro-substituted aromatic rings. In *chiral* drugs molecules containing CF_2 groups, the diastereotopic ^{19}F nuclei have different chemical shifts, if they are not too far from a stereogenic centre. In such cases, it is straightforward to excite and observe an LLS involving two ^{19}F spins. In our laboratory, we often use a sequence of non-selective (“hard”) pulses developed for this purpose (Sarkar et al., 2007). Indeed, it has been demonstrated that LLSs involving two ^{19}F spins can be readily observed in diastereotopic CF_2 groups (Buratto et al., 2016) and that their lifetimes T_{LLS} can exceed T_1 . In *achiral* molecules on the other hand, pairs of ^{19}F atoms attached to the same carbon are chemically equivalent, i.e., have degenerate chemical shifts, so that the geminal $^2J(^{19}\text{F}, ^{19}\text{F})$ couplings do not affect the spectrum to first order. Yet LLS can be excited in such systems, provided that the pairs of ^{19}F atoms are *magnetically inequivalent*. Two ^{19}F atoms attached to the same carbon atom are magnetically inequivalent if and only if the vicinal couplings $^3J(^{19}\text{F}, ^{19}\text{F})$ to neighbouring ^{19}F nuclei are not degenerate, i.e., provided differences such as $\Delta J_{\text{AM}} = J_{\text{AM}} - J_{\text{AM}'} = J_{\text{A'M'}} - J_{\text{A'M}}$ and $\Delta J_{\text{MX}} = J_{\text{MX}} - J_{\text{MX}'} = J_{\text{M'X'}} - J_{\text{M'X}}$ do not vanish. The degeneracy of vicinal scalar couplings is lifted provided the potential wells of the different rotamers that result from rotations about the C-C bonds are not equally populated.



This work extends the excitation of LLS by Spin-Lock Induced Crossing (SLIC) (DeVience et al., 2013) in a set of 4 or 6 ^{19}F spins in perfluorinated aliphatic chains of the type $-(\text{CF}_2)_n-$. Specifically, we have looked at per- and polyfluoroalkyl (PFAS) molecules, which have been widely studied for their detrimental effects on the environment (Fenton et al., 2021). We have used mono-chromatic and poly-chromatic SLIC, involving the application of 1, 2 or 3 radio-frequency (RF) fields simultaneously to 1, 2 or 3 multiplets in the ^{19}F spectrum (Fig. 1), in analogy to our work on ^1H spins in aliphatic chains of the type $-(\text{CH}_2)_n-$ (Sonnefeld et al., 2022a, b; Razanahoera et al., 2023; Sheberstov et al., 2024). The resulting long-lived states involve 2, 4 or - to a lesser extent - 6 ^{19}F spins. One must distinguish between two strategies: either single- or double-quantum SLIC (SQ- or DQ-SLIC). For DQ-SLIC to be efficient, the RF amplitudes v_{SLIC} of SLIC pulses must be equal to the geminal coupling $^2J(^{19}\text{F}, ^{19}\text{F})$ between two neighbouring ^{19}F spins. For SQ-SLIC, v_{SLIC} of must be twice as large.



Figure 1. (a) Experiment used to amplify the amplitudes of forbidden “Outer Singlet-Triplet Transitions” (OSTs) in conventional ^{19}F spectra. The radio-frequency (RF) amplitude v_{SLIC} and duration τ_{SLIC} can be optimized empirically to achieve the highest possible OST signal amplitudes (Sheberstov et al., 2019). (b) Pulse sequence used to study the excitation, relaxation, and reconversion of LLS of ^{19}F in perfluorinated achiral aliphatic chains. The transverse magnetization is excited by a ‘hard’ $(\pi/2)_x$ pulse, followed by the application of one, two or three selective RF fields (polychromatic SLIC pulses) applied simultaneously at the resonance frequencies (chemical shifts) of one, two or three consecutive CF_2 groups to convert the magnetization into a superposition of various LLS. Two optima result from level anti-crossings at the single-quantum condition (SQ LAC), or at the double-quantum condition (DQ LAC). The RF amplitudes must be twice the geminal coupling $v_{\text{SLIC}}^{\text{SQ}} = 2 \ ^2J(^{19}\text{F}, ^{19}\text{F})$ for SQ LAC, or equal to the geminal coupling $v_{\text{SLIC}}^{\text{DQ}} = \ ^2J(^{19}\text{F}, ^{19}\text{F})$ for DQ LAC. The maximum efficiency is achieved either for a short pulse duration $\tau_{\text{SLIC}}^{\text{SQ}} = 1/(\sqrt{2}\Delta J)$ for SQ LAC or a longer duration $\tau_{\text{SLIC}}^{\text{DQ}} = \sqrt{2}\tau_{\text{SLIC}}^{\text{SQ}} = 1/(\Delta J)$ for DQ LAC, where $\Delta J = (\Delta J_{\text{AM}} + \Delta J_{\text{MX}})/2$ and $\Delta J_{\text{AM}} = J_{\text{AM}} - J_{\text{AM}'} = J_{\text{A}'\text{M}'} - J_{\text{A}'\text{M}}$ and $\Delta J_{\text{AX}} = J_{\text{AX}} - J_{\text{AX}'} = J_{\text{A}'\text{X}'} - J_{\text{A}'\text{X}}$. After a T_{00} filter, another set of polychromatic SLIC pulses allows one to reconvert LLS into observable magnetization.

A population imbalance between the triplet and singlet states can also be obtained at very low spin temperatures, as may occur in dynamic nuclear polarization (DNP) (Tayler et al., 2012; Bornet et al., 2014; Razanahoera et al., 2024). In systems with more than two spins, one can also excite long-lived imbalances between states that belong to different symmetries of the spin permutation group (Kress et al., 2019).

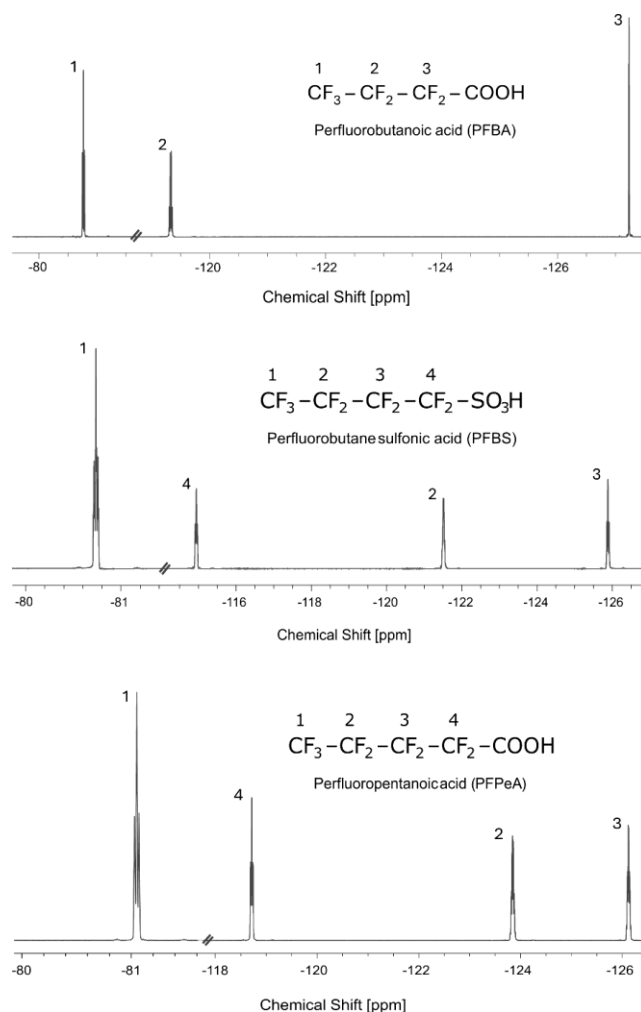


90 2 Results and Discussion

To extend the excitation of LLS from ^1H to ^{19}F , a challenge arises from the fact that $J(^{19}\text{F}-^{19}\text{F})$ couplings obey different rules compared to $J(^1\text{H}-^1\text{H})$ couplings. Typically, geminal $^2J(^{19}\text{F}-^{19}\text{F})$ couplings in CF_2 groups are on the order of 250 to 290 Hz (Krivdin, 2020), much larger than geminal $^2J(^1\text{H}-^1\text{H})$ couplings in CH_2 groups which are about ~ 15 Hz. In ^1H NMR, typical values of the difference between vicinal couplings lie in the range $0 < \Delta J(^1\text{H}) < 7$ Hz, while for ^{19}F NMR, these differences
95 may typically lie in the range $0 < \Delta J(^{19}\text{F}) < 40$ Hz.

We have explored three achiral fluorinated molecules shown in , selected because their chemical shifts cover a sufficient spread, so that their scalar couplings are weak compared to the shift differences (the first-order spectra fulfil the weak coupling approximation at 11.6 T, 470.46 MHz for ^{19}F , 500 MHz for ^1H). Strong coupling (as may occur at lower static fields, e.g., in
100 our 1.4 T spectrometer where ^{19}F can be observed at 58.69 MHz and ^1H at 60 MHz) leads to additional challenges. Note that if the molecules contain ^1H in addition to ^{19}F , it may be advisable to use ^1H decoupling during excitation of the LLS, their reconversion and the observation of the resulting ^{19}F signals (Buratto et al., 2016), although heteronuclear couplings may open alternative paths for the excitation of LLS.

105 Note that other popular drug screening methods using ligand-observed NMR that are widely used in pharmaceutical industry, such as Saturation Transfer Difference (STD) spectroscopy (Mayer and Meyer, 1999), or water-ligand observed via gradient spectroscopy (waterLOGSY) (Dalvit et al., 2000), cannot be adapted to ^{19}F since both methods require reservoirs of ^1H of the drug molecule, the target protein and the H_2O solvent.



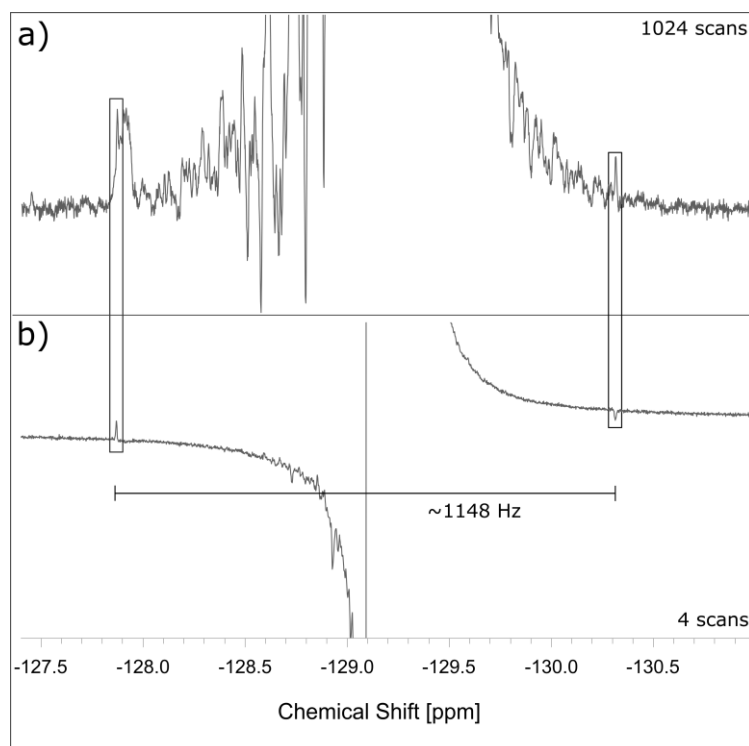
110 **Figure 2. Three achiral molecules with fluorinated aliphatic chains studied in this work: perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), and perfluoropentanoic acid (PFPeA). All three molecules were dissolved in DMSO-d₆ at 500 mM concentrations. The spectra were acquired at 298 K on a Bruker WB spectrometer at 11.6 T (470.46 MHz for ^{19}F , 500 MHz for ^1H) equipped with a NEO console. All spectra feature weak first-order scalar couplings at this field.**

2.1 Outer Singlet-Triplet Transitions

115 For the excitation of LLS in aliphatic chains containing ^1H , we have estimated geminal $^2J(^1\text{H}-^1\text{H})$ couplings and used spin simulation programs (Cheshkov et al., 2018) to optimize the radio-frequency field amplitude v_{SLIC} and the duration τ_{SLIC} . For ^{19}F , it is more difficult to estimate the relevant coupling constants. As a consequence of chemical equivalence, the geminal couplings $^2J(^{19}\text{F}, ^{19}\text{F})$ cannot be observed directly, but manifest themselves as weak forbidden combination lines (henceforth called “Outer Singlet-Triplet Transitions” or OSTs) that appear on either side of the ^{19}F multiplets with intensities that are ca.



120 10^4 times weaker than those of the allowed transitions. These OSTs are much harder to detect than the equivalent forbidden
 OSTs in ^1H spectra, where the amplitudes are on the order of 1 % of the allowed transitions because the scalar couplings are
 more favorable. The detection of OSTs in ^{19}F NMR can be improved (Sheberstov et al., 2019) by irradiating one of the
 multiplets with a single monochromatic RF field with an amplitude ν_{rf} in the vicinity of the optimum value ν_{SLIC} and a duration
 near the ideal τ_{SLIC} (Fig. 1a.) We were able to enhance the intensities of the OSTs by up to two orders of magnitude. In this
 125 work, we have in this manner measured geminal couplings that fall in the range of $280 < {}^2J(^{19}\text{F}, ^{19}\text{F}) < 295$ Hz. Once the
 amplitude ν_{SLIC} has been optimized, the duration τ_{SLIC} can readily be optimized by searching empirically for the highest signal
 amplitude.



130 **Figure 3. a) Conventional ^{19}F spectrum of the multiplet of the low-frequency CF_2 group (peak 3 in Figure 2) of 6.9 M**
perfluorobutanoic acid (PFBA) in DMSO- d_6 . The forbidden combination lines, known as Outer Singlet-Triplet transitions (OSTs),
are emphasized by rectangular frames, but cannot be reliably identified as such on the grounds of this spectrum alone. b) The OSTs
were enhanced by irradiation with an RF field with an amplitude $\nu_{\text{SLIC}} = 574$ Hz, applied to the centre of the low-frequency CF_2
group in PFBA during $\tau_{\text{SLIC}} 50$ ms, immediately followed by the observation of the ^{19}F free induction decay (i.e., without conversion
 135 **into LLS). The frequency difference between the two framed transitions is 1148 Hz, which corresponds to four times ${}^2J(^{19}\text{F}-^{19}\text{F})$. The**
top spectrum required 1024 scans, while the bottom spectrum was obtained in only 4 scans.



2.2 Lifetimes of Long-Lived States

The preliminary experiments shown in Fig. 3 allowed us to set up optimized conditions for SLIC. Using the pulse sequence of Fig. 1b, we observed the decay curves of Fig. 4.

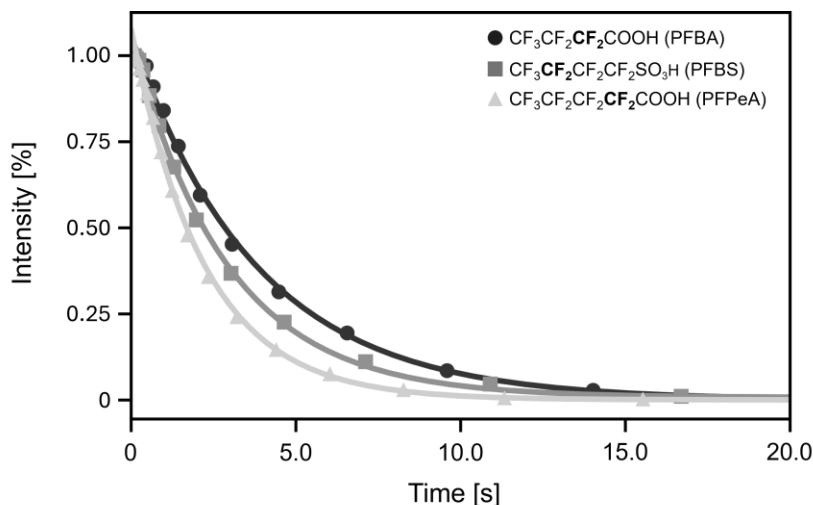


Figure 4. Decays of LLS (actually admixtures of two-, four-, and minor amounts of six-spin order LLS terms) for three different fluorinated molecules. The decays were fitted with mono-exponential functions to determine the LLS lifetimes reported in Table 1. The CF₂ groups for which the decay is shown are bold in the molecular formulae.

The resulting LLS lifetimes are reported in Table 1. Note that at a field of 11.6 T, the ratios T_{LLS}/T_1 all lie in the vicinity of 3. Fluorinated methyl CF₃ groups do not contribute to the LLS, as proven by selective decoupling at their resonance frequencies which affects neither the efficiency of LLS excitation nor their lifetimes. We shall demonstrate elsewhere how the relaxation times are affected by binding of the ligands to target proteins, and how the contrast can be improved by exploiting the field dependence.



Table 1: Optimized radio-frequency field amplitude v_{SLIC} and optimized duration τ_{SLIC} for Spin-Lock Induced Crossings (SLIC) to generate long-lives states involving 4 or 6 ^{19}F spins of the fluorinated aliphatic chains, using 2 or 3 RF fields applied simultaneously to the 2 or 3 multiplets of the three fluorinated molecules (Fig. 2). The spin-lattice relaxation times T_1 were determined by the inversion-recovery method. The relaxation times T_{LLS} were determined as described in Fig. 1b (Sonnefeld et al., 2022b), combined with a 4-step phase cycle. The errors correspond to one standard deviation. The sample of PFBA had a concentration of 70 mM, while the samples of PFBS and PFPeA both had concentrations of 500 mM in DMSO-d₆.

Compound	v_{SLIC} [Hz]	τ_{SLIC} [ms]	Peak	T_1 [s]	T_{LLS} [s]	Ratio T_{LLS}/T_1
Perfluorobutanoic acid (PFBA)	574	60	2	1.09 ± 0.01	3.64 ± 0.09	3.6
			3	1.78 ± 0.01	3.81 ± 0.11	2.1
Perfluorobutane sulfonic acid (PFBS)	576	35	2	1.26 ± 0.0	2.78 ± 0.07	2.2
			3	1.21 ± 0.0	2.92 ± 0.07	2.4
			4	1.27 ± 0.0	2.91 ± 0.07	2.3
Perfluoropentanoic acid (PFPeA)	585	80	2	0.69 ± 0.0	2.24 ± 0.06	3.2
			3	1.04 ± 0.0	2.23 ± 0.05	2.1
			4	1.09 ± 0.0	2.41 ± 0.07	2.2

3 Conclusion

It has been shown that for fluorinated aliphatic chains, forbidden Outer Singlet-Triplet transitions (OSTs) can be observed in one-dimensional ^{19}F NMR spectra, which is crucial for the optimization of SLIC parameters to create long-lived states involving 4 or 6 ^{19}F spins in isotropic solution. We demonstrate that long-lived states of ^{19}F spins can be readily excited in three different achiral molecules containing fluorinated aliphatic chains. At a field of 11.6 T (470.46 MHz for ^{19}F , 500 MHz for ^1H), the lifetimes $T_{\text{LLS}}(^{19}\text{F})$ of the long-lived states exceed the longitudinal relaxation times $T_1(^{19}\text{F})$ by about a factor 3. Fluorinated aliphatic chains can be attached to drugs to modify their metabolic or pharmacokinetic properties. This makes the excitation of LLS in these chains interesting for drug screening, where it has been previously demonstrated that LLS show good contrast with target proteins.

Author Contributions

K.S. designed the research. C.W. and S. D. performed the experiments and analysed the data. G.B. contributed to writing the report.



Conflict of Interest

Geoffrey Bodenhausen is a member of the editorial board of Magnetic Resonance Ampere.

180 The authors have no other competing interests to declare.

Financial Support

This work was supported by the European Research Council (ERC), Synergy grant “Highly Informative Drug Screening by Overcoming NMR Restrictions” (HISCORE, grant agreement number 951459). KS acknowledges support by l’Agence Nationale de la Recherche (ANR) for the project THROUGH-NMR (ANR-24-CE93-0011-01).

185 References

- Aguilar, J. A., Nilsson, M., Bodenhausen, G., and Morris, G. A.: Spin echo NMR spectra without J modulation, *Chem Commun*, 48, 811–813, <https://doi.org/10.1039/C1CC16699A>, 2012.
- Bornet, A., Ji, X., Mammoli, D., Vuichoud, B., Milani, J., Bodenhausen, G., and Jannin, S.: Long-Lived States of Magnetically Equivalent Spins Populated by Dissolution-DNP and Revealed by Enzymatic Reactions, *Chem. – Eur. J.*, 20, 17113–17118, <https://doi.org/10.1002/chem.201404967>, 2014.
- 190 Buchholz, C. R. and Pomerantz, W. C. K.: ^{19}F NMR viewed through two different lenses: ligand-observed and protein-observed ^{19}F NMR applications for fragment-based drug discovery, *RSC Chem. Biol.*, 2, 1312–1330, <https://doi.org/10.1039/D1CB00085C>, 2021.
- Buratto, R., Mammoli, D., Canet, E., and Bodenhausen, G.: Ligand–Protein Affinity Studies Using Long-Lived States of Fluorine-19 Nuclei, *J. Med. Chem.*, 59, 1960–1966, <https://doi.org/10.1021/acs.jmedchem.5b01583>, 2016.
- 195 Cavadini, S., Dittmer, J., Antonijevic, S., and Bodenhausen, G.: Slow Diffusion by Singlet State NMR Spectroscopy, *J. Am. Chem. Soc.*, 127, 15744–15748, <https://doi.org/10.1021/ja052897b>, 2005.
- Cheshkov, D. A., Sheberstov, K. F., Sinitsyn, D. O., and Chertkov, V. A.: ANATOLIA: NMR software for spectral analysis of total lineshape, *Magn. Reson. Chem.*, 56, 449–457, <https://doi.org/10.1002/mrc.4689>, 2018.
- 200 Dalvit, C., Pevarello, P., Tato, M., Veronesi, M., Vulpetti, A., and Sundström, M.: Identification of compounds with binding affinity to proteins via magnetization transfer from bulk water, n.d.
- DeVience, S. J., Walsworth, R. L., and Rosen, M. S.: Preparation of Nuclear Spin Singlet States Using Spin-Lock Induced Crossing, *Phys. Rev. Lett.*, 111, 173002, <https://doi.org/10.1103/PhysRevLett.111.173002>, 2013.
- Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., Smith, J. S., and Roberts, S. M.: Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing
- 205 Future Research, *Environ. Toxicol. Chem.*, 40, 606–630, <https://doi.org/10.1002/etc.4890>, 2021.



- Kiryutin, A. S., Rodin, B. A., Yurkovskaya, A. V., Ivanov, K. L., Kurzbach, D., Jannin, S., Guarin, D., Abergel, D., and Bodenhausen, G.: Transport of hyperpolarized samples in dissolution-DNP experiments, *Phys. Chem. Chem. Phys.*, 21, 13696–13705, <https://doi.org/10.1039/C9CP02600B>, 2019.
- 210 Kress, T., Walrant, A., Bodenhausen, G., and Kurzbach, D.: Long-Lived States in Hyperpolarized Deuterated Methyl Groups Reveal Weak Binding of Small Molecules to Proteins, *J. Phys. Chem. Lett.*, <https://doi.org/10.1021/acs.jpcllett.9b00149>, 2019.
- Krivdin, L. B.: Computational aspects of ^{19}F NMR, 2020.
- Lorz, N., Czarniecki, B., Loss, S., Meier, B., and Gossert, A. D.: Higher Contrast in ^1H -Observed NMR Ligand Screening with the PEARLScreen Experiment, *Angew. Chem. Int. Ed.*, 64, e202423879, <https://doi.org/10.1002/anie.202423879>, 2025.
- 215 Mayer, M. and Meyer, B.: Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy, *Angew. Chem. Int. Ed.*, 38, 1784–1788, [https://doi.org/10.1002/\(SICI\)1521-3773\(19990614\)38:12<1784::AID-ANIE1784>3.0.CO;2-Q](https://doi.org/10.1002/(SICI)1521-3773(19990614)38:12<1784::AID-ANIE1784>3.0.CO;2-Q), 1999.
- Pileio, G., Hill-Cousins, J. T., Mitchell, S., Kuprov, I., Brown, L. J., Brown, R. C. D., and Levitt, M. H.: Long-Lived Nuclear Singlet Order in Near-Equivalent ^{13}C Spin Pairs, *J. Am. Chem. Soc.*, 134, 17494–17497, <https://doi.org/10.1021/ja3089873>, 2012.
- 220 Razanahoera, A., Sonnefeld, A., Bodenhausen, G., and Sheberstov, K.: Paramagnetic relaxivity of delocalized long-lived states of protons in chains of CH_2 groups, *Magn. Reson.*, 4, 47–56, <https://doi.org/10.5194/mr-4-47-2023>, 2023.
- Razanahoera, A., Sonnefeld, A., Sheberstov, K., Narwal, P., Minaei, M., Kouřil, K., Bodenhausen, G., and Meier, B.: Hyperpolarization of Long-Lived States of Protons in Aliphatic Chains by Bullet Dynamic Nuclear Polarization, Revealed on the Fly by Spin-Lock-Induced Crossing, *J. Phys. Chem. Lett.*, 15, 9024–9029, <https://doi.org/10.1021/acs.jpcllett.4c01457>, 2024.
- Salvi, N., Buratto, R., Bornet, A., Ulzega, S., Rentero Rebollo, I., Angelini, A., Heinis, C., and Bodenhausen, G.: Boosting the Sensitivity of Ligand–Protein Screening by NMR of Long-Lived States, *J. Am. Chem. Soc.*, 134, 11076–11079, <https://doi.org/10.1021/ja303301w>, 2012.
- 230 Sarkar, R., Vasos, P. R., and Bodenhausen, G.: Singlet-State Exchange NMR Spectroscopy for the Study of Very Slow Dynamic Processes, *J. Am. Chem. Soc.*, 129, 328–334, <https://doi.org/10.1021/ja0647396>, 2007.
- Shah, P. and Westwell, A. D.: The role of fluorine in medicinal chemistry: Review Article, *J. Enzyme Inhib. Med. Chem.*, 22, 527–540, <https://doi.org/10.1080/14756360701425014>, 2007.
- Sheberstov, K. F., Kiryutin, A. S., Bengs, C., Hill-Cousins, J. T., Brown, L. J., Brown, R. C. D., Pileio, G., Levitt, M. H., Yurkovskaya, A. V., and Ivanov, K. L.: Excitation of singlet–triplet coherences in pairs of nearly-equivalent spins, *Phys. Chem. Chem. Phys.*, 21, 6087–6100, <https://doi.org/10.1039/C9CP00451C>, 2019.
- 235 Sheberstov, K. F., Sonnefeld, A., and Bodenhausen, G.: Collective long-lived zero-quantum coherences in aliphatic chains, *J. Chem. Phys.*, 160, 144308, <https://doi.org/10.1063/5.0196808>, 2024.
- Sonnefeld, A., Razanahoera, A., Pelupessy, P., Bodenhausen, G., and Sheberstov, K.: Long-lived states of methylene protons in achiral molecules, *Sci. Adv.*, 8, eade2113, <https://doi.org/10.1126/sciadv.ade2113>, 2022a.
- 240



- Sonnefeld, A., Bodenhausen, G., and Sheberstov, K.: Polychromatic Excitation of Delocalized Long-Lived Proton Spin States in Aliphatic Chains, *Phys. Rev. Lett.*, 129, 183203, <https://doi.org/10.1103/PhysRevLett.129.183203>, 2022b.
- Takegoshi, K., Ogura, K., and Hikichi, K.: A perfect spin echo in a weakly homonuclear J-coupled two spin- system, *J. Magn. Reson.* 1969, 84, 611–615, [https://doi.org/10.1016/0022-2364\(89\)90127-3](https://doi.org/10.1016/0022-2364(89)90127-3), 1989.
- 245 Tayler, M. C. D., Marco-Rius, I., Kettunen, M. I., Brindle, K. M., Levitt, M. H., and Pileio, G.: Direct Enhancement of Nuclear Singlet Order by Dynamic Nuclear Polarization, *J. Am. Chem. Soc.*, 134, 7668–7671, <https://doi.org/10.1021/ja302814e>, 2012.
- Vasos, P. R., Comment, A., Sarkar, R., Ahuja, P., Jannin, S., Ansermet, J.-P., Konter, J. A., Hautle, P., van den Brandt, B., and Bodenhausen, G.: Long-lived states to sustain hyperpolarized magnetization, *Proc. Natl. Acad. Sci.*, 106, 18469–18473, <https://doi.org/10.1073/pnas.0908123106>, 2009.