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

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Abstract. *Long-lived states* (LLS) have lifetimes T_{LLS} that exceed longitudinal spin-lattice relaxation times T_1. In this study, lifetimes T_{LLS} (^{19}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~~7 T, the lifetimes T_{LLS}(^{19}F) exceed the longitudinal relaxation times T_1(^{19}F) by about a factor 3. The lifetimes T_{LLS}(^{19}F) can be strongly affected by binding to macromolecules, a feature that can be exploited for screening of fluorinated drugs. ~~Both T_{LLS}(^{19}F) and T_1(^{19}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 ^{1}H, and more rarely, for 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).~~

The discovery of long-lived states (LLS) was inspired by spin isomers of dihydrogen (H_2), in which a nonequilibrium ratio of para- vs ortho-dihydrogen can persist for many days before relaxing. Many applications of LLS have been illustrated for pairs of ^{13}C or ^{15}N nuclei (Pileio et al., 2012a; Feng et al., 2013; Stevanato et al., 2015; Elliott et al., 2019; Sheberstov et al., 2019b), for pairs of ^{1}H nuclei (Franzoni et al., 2012; Kiryutin et al., 2019a), and less frequently, for pairs of ^{19}F nuclei (Buratto et al., 2016), for ^{31}P (Korenchan et al., 2021), and for ^{103}Rh spins (Harbor-Collins et al., 2024). 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., 2012b; Kiryutin et al., 2019b), 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). Another

application of LLS is spectral editing, in particular for selective filtering of selected signals in proteins (Mamone et al., 2020; Sicoli et al., 2024).

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 ^{1}H NMR, while the absence of background signals offers decisive advantages~~Positron emission tomography (PET) with ^{18}F-labeled radioligands is widely applied in diagnosis and in drug development (Nerella et al., 2022). 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 ^{1}H NMR, while signal overlap is significantly reduced due to the greater chemical shift dispersion, and spectral quality is improved because of the absence of background signals, e.g., water signal (Buchholz and Pomerantz, 2021). For drug 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.~~A LLS involving two or more ^{19}F spins in a ligand can lead to a remarkable contrast upon binding to a protein target (Buratto et al., 2016). For ligands that bind to a target protein, contrast in ^{19}F NMR arises from the combined effects of binding on the chemical shifts, on the reduction of symmetry when achiral ligands bind to chiral targets, 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 $^{n}J(^{19}\text{F}, ^{19}\text{F})$, 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 latter two effects can be distinguished by comparing Carr-Purcell-Meiboom-Gill (CPMG) echo trains with high repetition rates and slow CPMG echo trains using so-called "perfect echoes". The first type of experiment will suppress echo modulations due to homonuclear scalar couplings $^{n}J(^{19}\text{F}, ^{19}\text{F})$ and inhibit echo decays due to intermediate exchange (Takegoshi et al., 1989; Aguilar et al., 2012), while the second type of experiment eliminates the effects of $^{n}J(^{19}\text{F}, ^{19}\text{F})$ 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).~~(Lorz et al., 2025).

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 $\langle p(T^{AA'})\rangle = \frac{1}{3}\left(p(T_{+1}^{AA'}) + p(T_0^{AA'}) + p(T_{-1}^{AA'})\right)$ is known as *triplet-singlet population imbalance*, which

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is immune against relaxation driven by intra-pair dipole-dipole couplings ~~(Sonnefeld et al., 2022a). Such~~ . One speaks of LLSs since these population imbalances ~~are also known as LLSs since their~~decay with lifetimes T_{LLS} that can greatly exceed T_1 and T_2. Such ~~states~~a state can be described by a scalar ~~products of the type~~product $\hat{I}^A \cdot \hat{I}^{A'}$, where $\hat{I}^p = (\hat{I}_x^p, \hat{I}_y^p, \hat{I}_z^p),\ p \in \{A, A'\}$. In an achiral aliphatic chain with 6 spins, ~~described by~~denoted to 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 ~~$\hat{I}^X \cdot \hat{I}^{X'}$~~ $\hat{I}^X \cdot \hat{I}^{X'}$ but also 4-spin order terms such as ~~$\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^M \cdot \hat{I}^{M'} (\hat{I}^A \cdot \hat{I}^{A'}) \cdot (\hat{I}^M \cdot \hat{I}^{M'})$~~, and/or $(\hat{I}^A \cdot \hat{I}^{A'}) \cdot (\hat{I}^X \cdot \hat{I}^{X'})$ and/or ~~$\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$ and/or $\hat{I}^M \cdot \hat{I}^{M'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$.~~ $(\hat{I}^M \cdot \hat{I}^{M'}) \cdot (\hat{I}^X \cdot \hat{I}^{X'})$. One can also, in principle, excite a 6-spin order term ~~$\hat{I}^A \cdot \hat{I}^{A'} \cdot \hat{I}^M \cdot \hat{I}^{M'} \cdot \hat{I}^X \cdot \hat{I}^{X'}$,~~ $(\hat{I}^A \cdot \hat{I}^{A'}) \cdot (\hat{I}^M \cdot \hat{I}^{M'}) \cdot (\hat{I}^X \cdot \hat{I}^{X'})$, although simulations for ~~protons in~~protonated aliphatic chains indicate ~~a low yield of~~that the ~~excitation~~yields of such a 6-spin term ~~are low (Sonnefeld et al., 2022a).~~ It is challenging to determine separately the coefficients if one excites admixtures of all seven possible products in a 6-spin system ~~since one usually creates admixtures.~~ Since experiments indicate that ~~their~~the decay constants are often very similar, admixtures of different products tend to relax with an effective mono-exponential ~~decays~~decay. These remarks apply to ~~many~~ molecules containing either ~~^{19}F~~three fluorinated or ~~^{1}H nuclei~~three protonated methylene groups, including those analyzed in this study and in our previous work on protons (Sonnefeld et al., 2022a).

In ~~some~~many molecules, there are two ^{19}F spins that 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 the two ^{19}F spins ~~of a CF_2 group.~~ In our laboratory, we often use a sequence of ~~non-selective ("~~hard~~")~~ pulses developed for this purpose (Sarkar et al., 2007). ~~Indeed, it has~~LLSs have thus been ~~demonstrated that LLSs involving two ^{19}F spins can be readily~~ observed in diastereotopic CF_2 groups where their lifetimes T_{LLS} exceed T_1 significantly (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}F, ^{19}F) couplings ~~do~~does not affect the ~~spectrum~~spectra to first order. Yet LLS can be excited in such systems~~,~~ provided that the pairs of ^{19}F atoms are *magnetically inequivalent*. ~~Two~~A pair of ^{19}F atoms attached to the same carbon atom are magnetically inequivalent if and only if ~~the~~ vicinal couplings such as ^3J(^{19}F, ^{19}F~~)~~, or ^3J(^{19}F, ^{1}H), to ^{19}F or ^{1}H nuclei in neighbouring ~~^{19}F nuclei~~CF_2 or CH_2 groups are *not* degenerate, i.e., provided differences such as $\Delta J_{AM} = J_{AM} - J_{AM'} = J_{A'M'} - J_{A'M}$ and $\Delta J_{MX} = J_{MX} - J_{MX'} = J_{M'X'} - J_{M'X}$ do not vanish. The degeneracy of vicinal scalar couplings is lifted ~~provided~~when the ~~potential wells of the different~~ rotamers ~~that result from~~produced by rotations about the C–C bonds are not equally populated. This occurs if the potential wells corresponding to the different rotamers have unequal energy.

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This work extends the excitation of LLS by Spin-Lock Induced Crossing (SLIC) (DeVience et al., 2013) ~~in~~to a set of 4 or 6 ^{19}F spins in perfluorinated aliphatic chains of the type $-(CF_2)_n-$. 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~~Studying these molecules and their ability to bind to protein targets could shed more light on their impact on living organisms.

Excitation with a SLIC pulse can also enhance the intensity of Outer Singlet–Triplet (OST) coherences (Sheberstov et al., 2019a), as discussed in more detail below. We have used mono- and poly-chromatic SLIC, involving the simultaneous application of 1, 2 or 3 radio-frequency (RF) fields to 1, 2 or 3 multiplets in a ^{19}F spectrum (Fig. 1), in analogy to our work on ^{1}H spins in aliphatic chains of the type –$(CH_2)_n$– ~~(Sonnefeld et al., 2022a, b; Razanahoera et al., 2023; Sheberstov et al., 2024)~~(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~~approaches: single- or double-quantum SLIC (SQ- or DQ-SLIC). For DQ-SLIC to be efficient, the RF amplitudes ν_{SLIC} of SLIC pulses must be equal to the geminal coupling $^{2}J(^{19}F, ^{19}F)$ between two neighbouring ^{19}F spins~~. For SQ-SLIC, ν_{SLIC} of must be~~, or twice as large for SQ-SLIC.

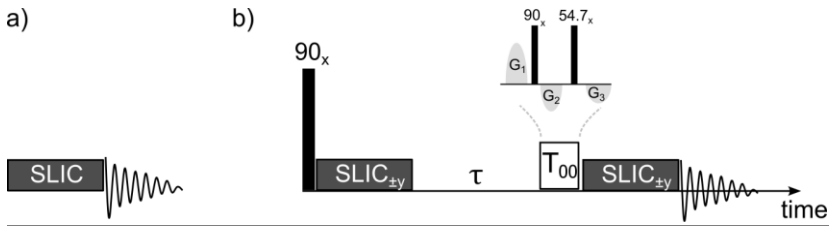

Figure 1. (a) Experiment used to amplify the amplitudes of forbidden "Outer Singlet-Triplet Transitions" (OSTs) in conventional ^{19}F spectra. The radio-frequency (RF) amplitude ν_{SLIC} and duration τ_{SLIC} can be optimized empirically to achieve the highest possible OST signal amplitudes ~~(Sheberstov 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'~~(Sheberstov et al., 2019a). (b) Pulse sequence used to study the excitation, relaxation, and reconversion of LLS of ^{19}F in fluorinated achiral aliphatic chains. The transverse magnetization is excited by a 'hard' non-selective $(\pi/2)_x$ 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 the 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 $\nu_{SLIC}^{SQ} = 2\,^{2}J(^{19}F, ^{19}F)$ for SQ LAC, or~~ equal to the geminal coupling ~~$\nu_{SLIC}^{DQ} = {}^{2}J(^{19}F, ^{19}F)$~~ for DQ LAC~~.~~, i.e., $\nu_{SLIC}^{DQ} = {}^{2}J(^{19}F, ^{19}F)$, or twice as large for SQ LAC. The maximum efficiency is achieved either for a short pulse duration $\tau_{SLIC}^{SQ} = 1/(|\sqrt{2}\Delta J|)$ for SQ LAC, or a longer duration $\tau_{SLIC}^{DQ} = \sqrt{2}\tau_{SLIC}^{SQ} = 1/(|\Delta J|)$ for DQ LAC, where $\Delta J = (\Delta J_{AM} + \Delta J_{MX})/2$ and $\Delta J_{AM} = J_{AM} - J_{AM'} = J_{A'M'} - J_{A'M}$ and $\Delta J_{AX} = J_{AX} - J_{AX'} = J_{A'X'} - J_{A'X}$. After a T_{00} filter~~, another set of polychromatic~~ (Tayler, 2020), another set of mono- or poly-chromatic SLIC pulses allows one to reconvert LLS into observable magnetization.

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~~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~~

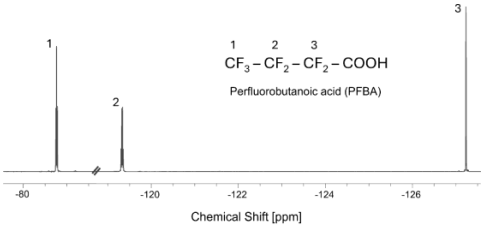
A population imbalance between the triplet and singlet states can also be obtained at very low spin temperatures, as may occur in dynamic nuclear polarization (DNP) (Tayler et al., 2012; Bornet et al., 2014; Razanahoera et al., 2024). In systems with more than two spins, one can also excite long-lived imbalances between states that belong to different symmetries of the spin permutation group, e.g. an imbalance between populations associated with irreducible representations A and E in CD_3 groups (Kress et al., 2019).

2 Results and Discussion

To extend the excitation of LLS from ^{1}H to ^{19}F, a challenge arises from the fact that $J(^{19}F\text{-}^{19}F)$ ~~couplings obey different rules compared to $J(^{1}H\text{-}^{1}H)$ couplings.~~and $J(^{1}H\text{-}^{1}H)$ differ in the way their magnitude depend on the number of chemical bonds between atoms. Typically, geminal $^{2}J(^{19}F\text{-}^{19}F)$ couplings in CF_2 groups are on the order of 250 to 290 Hz ~~(Krivdin, 2020)~~(Krivdin, 2020), much larger than geminal $^{2}J(^{1}H\text{-}^{1}H)$ couplings in CH_2 groups which are about ~ -15 Hz. In ^{1}H NMR, typical values of the difference between vicinal couplings lie in the range $0 < \Delta J(^{1}H) < 7$ Hz, while for ^{19}F NMR, these differences may typically lie in the range $0 < \Delta J(^{19}F) < 40$ 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 ^{1}H). Strong coupling (as may occur at lower static fields, e.g., in our 1.4 T spectrometer where ^{19}F can be observed at 58.69 MHz and ^{1}H at 60 MHz) leads to additional challenges. Note that if the molecules contain ^{1}H in addition to ^{19}F, it may be advisable to use ^{1}H 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.~~

~~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 ^{1}H of the drug molecule, the target protein and the H_2O solvent.~~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CF3 – CF2 – CF2 – COOH
Perfluorobutanoic acid (PFBA)
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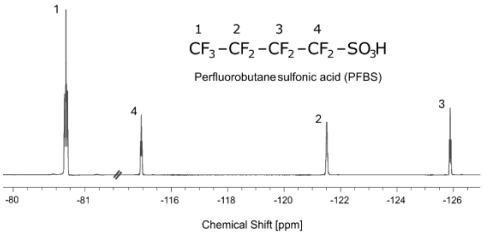
Chemical Shift [ppm]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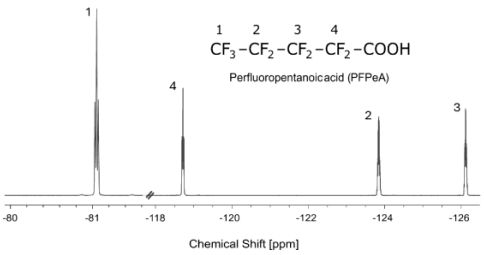
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CF3 – CF2 – CF2 – CF2 – COOH
Perfluoropentanoic acid (PFPeA)
-80
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Chemical Shift [ppm]

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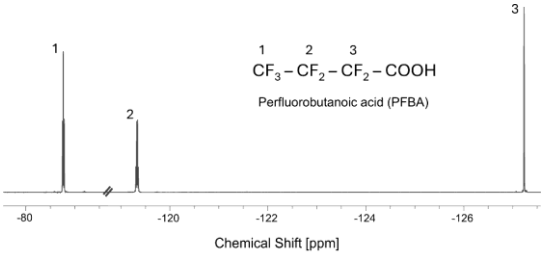

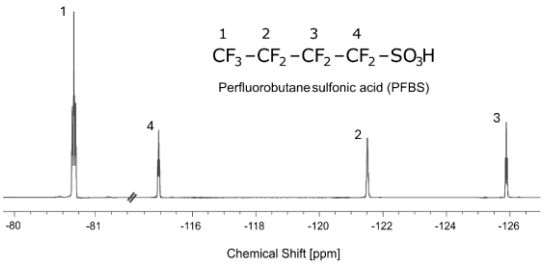

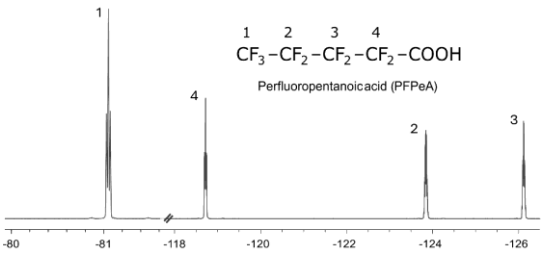

, selected because the chemical shifts between neighboring CF_2 groups exceed 1 kHz. This means that LLS excitation of a chosen CF_2 group is sufficiently selective, even with RF amplitudes on the order of 250 to 290 Hz for DQ LAC, or on the order of 500 to 580 Hz for SQ LAC respectively, so that one can neglect the effects of a SLIC pulse on neighboring CF_2 groups.

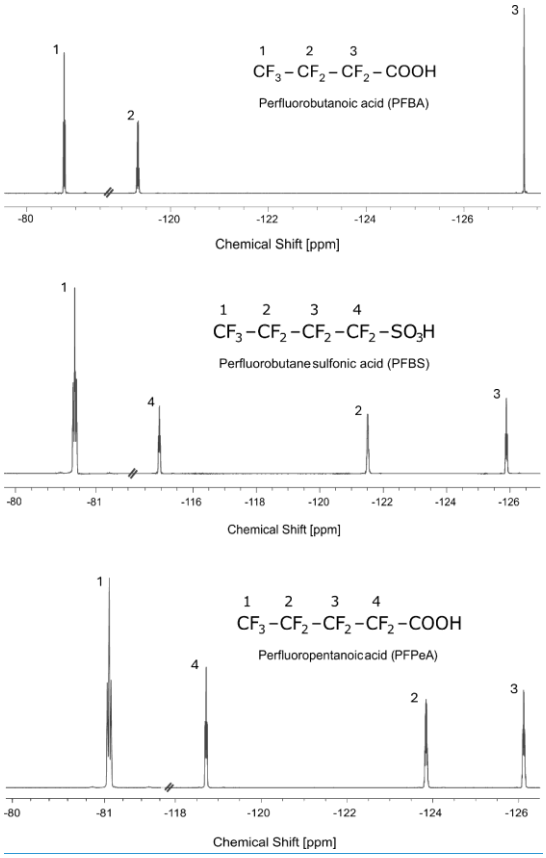

Figure 2. Three achiral molecules with fluorinated aliphatic chains studied in this work: perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), and perfluoropentanoic acid (PFPeA). All three molecules were dissolved in DMSO-d6 at 500 mM concentrations. The spectra were acquired at 298 K on a Bruker WB spectrometer at 11.67 T (470.46 MHz for ^{19}F, 500 MHz for ^{1}H) equipped with a NEO console~~. All spectra feature weak first-order scalar couplings at this field~~.

2.1 Outer Singlet-Triplet Transitions

For the excitation of LLS in aliphatic chains containing ^{1}H (Sonnefeld et al., 2022b), we have estimated the geminal $^{2}J(^{1}\text{H}\text{-}^{1}\text{H})$ couplings and used spin simulation programs (Cheshkov et al., 2018) to optimize the radio-frequency field amplitude ν_{SLIC} and the duration τ_{SLIC}. For ^{19}F, it is more difficult to estimate all relevant coupling constants. As a consequence of chemical

equivalence, the geminal couplings ^2J (^{19}F, ^{19}F) cannot be observed as a splitting, but manifest themselves through weakly allowed combination lines that appear on either side of the ^{19}F multiplets with intensities that are typically 10^4 times weaker than those of the allowed transitions. The detection of the OST transitions can be improved (Sheberstov et al., 2019a) 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.) Thus, we were able to enhance the intensities of the OST transitions by up to two orders of magnitude. In this 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. An enhancement of OST transitions can also be achieved using other techniques, such as J-synchronized CPMG (Sheberstov et al., 2019a) or symmetry-based sequences (Sabba et al., 2022).

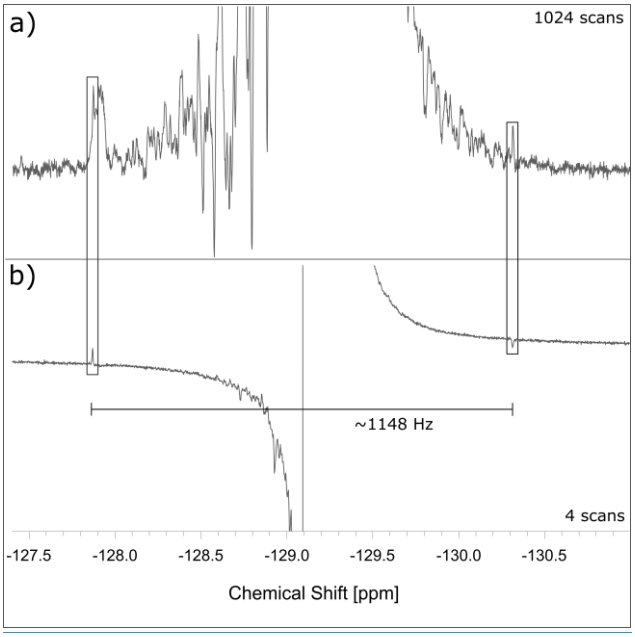

Figure 3. a) Conventional ^{19}F spectrum at 11.7 T (470.46 MHz for ^{19}F) of the multiplet of the low-frequency CF_2 group (peak 3 in Figure 2) of 6.9 M perfluorobutanoic acid (PFBA) in DMSO-d6. The ~~forbidden~~weakly allowed combination lines, known as Outer Singlet-Triplet transitions (OSTs), are emphasized by rectangular frames, but cannot be reliably identified as such on the grounds of ~~this~~the top spectrum alone. b) The OSTs were enhanced by irradiation with an RF field with an amplitude ν_{SLIC} = 574 Hz, applied to the centre of the low-frequency CF_2 group in PFBA during τ_{SLIC} 50 ms, immediately followed by the observation of the ^{19}F free induction decay (i.e., without conversion into LLS). The frequency difference between the two framed transitions is 1148 Hz, which

corresponds to four times $^2J(^{19}F\text{-}^{19}F)$. The top spectrum required 1024 scans, while the bottom spectrum was obtained ~~in~~with only 4 scans.

2.2 Lifetimes of Long-Lived States

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

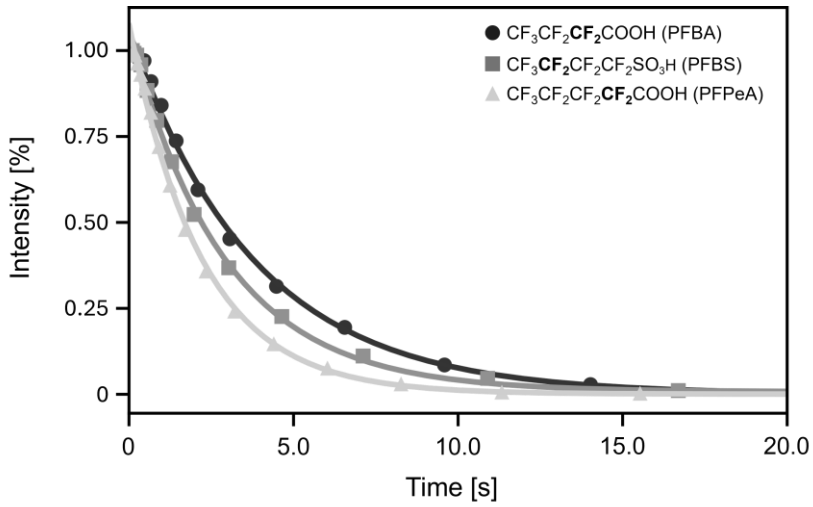

Figure 4. Decays of LLS (actually admixtures of two-, four-, and minor amounts of six-spin order LLS terms) for three different fluorinated molecules~~.~~ at 11.7 T (470.46 MHz for ^{19}F). The decays were fitted with mono-exponential functions to determine the LLS lifetimes reported in Table 1. The CF_2 groups for which the ~~decay is~~decays are shown are highlighted in bold in the molecular formulae.

The resulting LLS lifetimes are reported in Table 1. Note that at a field of 11.~~6~~7 T, the ratios T_{LLS}/T_1 all lie in the vicinity of 3. Fluorinated CF_3 methyl ~~CF_3~~ 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.~~of CF_2 groups nor their lifetimes.

Table 1: Optimized radio-frequency field amplitude ν_{SLIC} and optimized duration τ_{SLIC} for Spin-Lock Induced Crossings (SLIC) to generate long-~~lives~~lived 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).~~2) at 11.7 T (470.46 MHz for ^{19}F). 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.~~1b combined with a 4-step phase cycle (Sonnefeld et al., 2022b). The errors correspond to one standard deviation determined from the fitting procedure. The sample of PFBA had a concentration of 70 mM, while the samples of PFBS and PFPeA both had concentrations of 500 mM, all in DMSO-d6.

Compound	ν_{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

2.3 Protein-Ligand Titration of PFBS with BSA

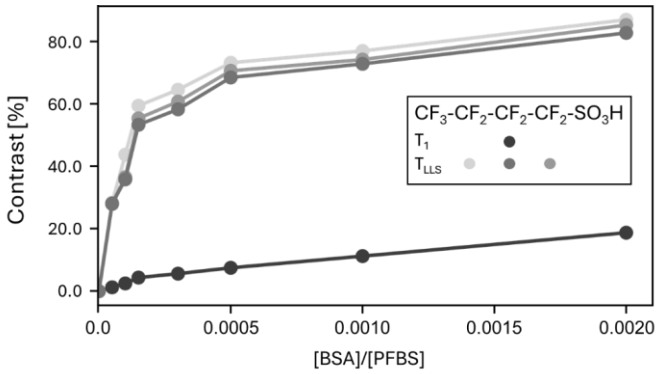

Figure 5. T_{LLS} and T_1 contrast curves for eight samples with [PFBS] = 50 mM, and 0 < [BSA] < 100 µM. The curves showing T_{LLS} contrast are shown for each of the CF_2 groups while the T_1 contrast is only shown for the central CF_2 group. The samples are composed of PFBS, BSA and phosphate buffer to obtain solutions of pH ≈ 7.2.

It has been previously reported that PFAS have a binding affinity for various proteins (Zhao et al., 2023). To evaluate the ability of ^{19}F LLS to provide contrast between a free and bound form, when interacting with a protein, we explored the binding of PFBS to the protein Bovine Serum Albumin (BSA), by measuring T_{LLS} of samples with [PFBS] = 50 mM and variating concentrations of 0 < [BSA] < 100 µM. The contrast can be defined as usual by $C_{LLS} = (R^{obs}_{LLS} - R^{free}_{LLS}) / R^{obs}_{LLS}$.

As shown in Fig. 5, the relaxation times, T_{LLS}, are clearly affected by binding of the ligand to the target protein. In addition, ^{19}F LLSs created in PFBS offer good contrast even if the protein is 10^{-4} times more dilute than the ligand. Note that the contrast obtained with $T_1(^{19}F)$ is much worse than the contrast obtained with T_{LLS} (^{19}F). We believe that the contrast can be improved at lower magnetic fields (where the chemical shift anisotropy is less efficient) but have not yet been able to verify this expectation at 1.4 T (56.5 MHz for ^{19}F, 60 MHz for ^{1}H) because of undesirable effects of selective RF fields on neighboring resonances in crowded spectra.

3 Conclusion

It has been shown that for fluorinated aliphatic chains, the amplitudes of forbidden Outer Singlet-Triplet transitions (OSTs) can be ~~observed~~boosted 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~~7~~ T (470.46 MHz for ^{19}F, 500 MHz for ^{1}H), the lifetimes $T_{LLS}(^{19}F)$ of the long-lived states exceed the longitudinal relaxation times $T_1(^{19}F)$ by ~~about~~ a factor between 2.2 and 3.6. Fluorinated aliphatic chains can be attached to existing drugs ~~to modify their~~that have

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~~when binding to target proteins.

Author Contributions

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

Conflict of Interest

~~Geoffrey Bodenhausen~~G.B is a member of the editorial board of Magnetic Resonance of the Groupement Ampere. The peer-review process was guided by an independent editor, and the authors have ~~also~~ no other competing interests to declare.

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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.~~

~~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.~~

~~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.~~

~~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 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.

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.jpclett.9b00149, 2019.

Krivdin, L. B.: Computational aspects of 19F 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.

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, 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 13C Spin Pairs, J. Am. Chem. Soc., 134, 17494–17497, https://doi.org/10.1021/ja3089873, 2012.

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.jpclett.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.

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.

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.

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, 1989.

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.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.

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.

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.

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.

Dumez, J.-N., Hill-Cousins, J. T., Brown, R. C. D., and Pileio, G.: Long-lived localization in magnetic resonance imaging, J. Magn. Reson. San Diego Calif 1997, 246, 27–30, https://doi.org/10.1016/j.jmr.2014.06.008, 2014.

Elliott, S. J., Kadeřávek, P., Brown, L. J., Sabba, M., Glöggler, S., O'Leary, D. J., Brown, R. C. D., Ferrage, F., and Levitt, M. H.: Field-cycling long-lived-state NMR of 15N2 spin pairs, Mol. Phys., 117, 861–867, https://doi.org/10.1080/00268976.2018.1543906, 2019.

Feng, Y., Theis, T., Liang, X., Wang, Q., Zhou, P., and Warren, W. S.: Storage of Hydrogen Spin Polarization in Long-Lived 13C2 Singlet Order and Implications for Hyperpolarized Magnetic Resonance Imaging, J. Am. Chem. Soc., 135, 9632–9635, https://doi.org/10.1021/ja404936p, 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 Future Research, Environ. Toxicol. Chem., 40, 606–630, https://doi.org/10.1002/etc.4890, 2021.

Franzoni, M. B., Buljubasich, L., Spiess, H. W., and Münnemann, K.: 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, 10393–10396, https://doi.org/10.1021/ja304285s, 2012.

Harbor-Collins, H., Sabba, M., Bengs, C., Moustafa, G., Leutzsch, M., and Levitt, M. H.: NMR spectroscopy of a 18O-labeled rhodium paddlewheel complex: Isotope shifts, 103Rh–103Rh spin–spin coupling, and 103Rh singlet NMR, J. Chem. Phys., 160, 014305, https://doi.org/10.1063/5.0182233, 2024.

Kiryutin, A. S., Panov, M. S., Yurkovskaya, A. V., Ivanov, K. L., and Bodenhausen, G.: Proton Relaxometry of Long-Lived Spin Order, ChemPhysChem, 20, 766–772, https://doi.org/10.1002/cphc.201800960, 2019a.

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, 2019b.

Korenchan, D. E., Lu, J., Levitt, M. H., and Jerschow, A.: 31P nuclear spin singlet lifetimes in a system with switchable magnetic inequivalence: experiment and simulation, Phys. Chem. Chem. Phys., 23, 19465–19471, https://doi.org/10.1039/D1CP03085J, 2021.

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.jpclett.9b00149, 2019.

Krivdin, L. B.: Computational aspects of ^{19} F NMR, Russ. Chem. Rev., 89, 1040–1073, https://doi.org/10.1070/RCR4948, 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.

Mamone, S., Rezaei-Ghaleh, N., Opazo, F., Griesinger, C., and Glöggler, S.: Singlet-filtered NMR spectroscopy, Sci. Adv., 6, eaaz1955, https://doi.org/DOI:%252010.1126/sciadv.aaz1955, 2020.

Melchiorre, G., Giustiniano, F., Rathore, S., and Pileio, G.: Singlet-assisted diffusion-NMR (SAD-NMR): extending the scope of diffusion tensor imaging via singlet NMR, Front. Chem., 11, 1224336, https://doi.org/10.3389/fchem.2023.1224336, 2023.

Nerella, S. G., Singh, P., Sanam, T., and Digwal, C. S.: PET Molecular Imaging in Drug Development: The Imaging and Chemistry Perspective, Front. Med., 9, https://doi.org/10.3389/fmed.2022.812270, 2022.

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 13C Spin Pairs, J. Am. Chem. Soc., 134, 17494–17497, https://doi.org/10.1021/ja3089873, 2012a.

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 13C Spin Pairs, J. Am. Chem. Soc., 134, 17494–17497, https://doi.org/10.1021/ja3089873, 2012b.

Pileio, G., Dumez, J.-N., Pop, I.-A., Hill-Cousins, J. T., and Brown, R. C. D.: Real-space imaging of macroscopic diffusion and slow flow by singlet tagging MRI, J. Magn. Reson., 252, 130–134, https://doi.org/10.1016/j.jmr.2015.01.016, 2015.

Pileio, G. and Ostrowska, S.: Accessing the long-time limit in diffusion NMR: The case of singlet assisted diffusive diffraction *q*-space, J. Magn. Reson., 285, 1–7, https://doi.org/10.1016/j.jmr.2017.10.003, 2017.

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.jpclett.4c01457, 2024.

Sabba, M., Wili, N., Bengs, C., Whipham, J. W., Brown, L. J., and Levitt, M. H.: Symmetry-based singlet–triplet excitation in solution nuclear magnetic resonance, J. Chem. Phys., 157, 134302, https://doi.org/10.1063/5.0103122, 2022.

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.

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, 2019a.

Sheberstov, K. F., Vieth, H.-M., Zimmermann, H., Rodin, B. A., Ivanov, K. L., Kiryutin, A. S., and Yurkovskaya, A. V.: Generating and sustaining long-lived spin states in 15N,15N′-azobenzene, Sci. Rep., 9, 20161, https://doi.org/10.1038/s41598-019-56734-y, 2019b.

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.

Sicoli, G., Sieme, D., Overkamp, K., Khalil, M., Backer, R., Griesinger, C., Willbold, D., and Rezaei-Ghaleh, N.: Large dynamics of a phase separating arginine-glycine-rich domain revealed via nuclear and electron spins, Nat. Commun., 15, 1610, https://doi.org/10.1038/s41467-024-45788-w, 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.

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.

Stevanato, G., Hill-Cousins, J. T., Håkansson, P., Roy, S. S., Brown, L. J., Brown, R. C. D., Pileio, G., and Levitt, M. H.: A Nuclear Singlet Lifetime of More than One Hour in Room-Temperature Solution, Angew. Chem. Int. Ed., 54, 3740–3743, https://doi.org/10.1002/anie.201411978, 2015.

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, 1989.

Tayler, M. C. D.: Filters for Long-lived Spin Order, https://doi.org/10.1039/9781788019972-00188, 2020.

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.

Zhao, L., Teng, M., Zhao, X., Li, Y., Sun, J., Zhao, W., Ruan, Y., Leung, K. M. Y., and Wu, F.: Insight into the binding model of per- and polyfluoroalkyl substances to proteins and membranes, Environ. Int., 175, 107951, https://doi.org/10.1016/j.envint.2023.107951, 2023.

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