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Long-Lived States in Hyperpolarized Deuterated Methyl Groups Reveal Weak Binding of Small Molecules to Proteins

Thomas Kress,^{†,‡} Astrid Walrant,[‡] Geoffrey Bodenhausen,^{*,†} and Dennis Kurzbach^{*,‡}

[†]Laboratoire des Biomolécules, LBM, Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France

[‡]Laboratoire des Biomolécules, LBM, École Normale Supérieure, Sorbonne Université, École Normale Supérieure, PSL University, CNRS, 75005 Paris, France

^{*}Faculty of Chemistry, Institute of Biological Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria

Supporting Information

ABSTRACT: We introduce a method for the detection of weak interactions of small molecules such as metabolites or medicaments that contain deuterated methyl groups with proteins in solution. The technique relies on long-lived imbalances of spin state populations, which are generated by dissolution dynamic nuclear polarization (D-DNP) and feature lifetimes that depend on the frequency of internal rotation of deuterated methyl groups. We demonstrate the technique for interactions between deuterated dimethyl sulfoxide (DMSO-*d*₆) and bovine serum albumin (BSA) or trypsin, where the methyl group rotation is slowed down upon protein binding, which causes a marked reduction in the lifetime of the population imbalances.

Deuterated drugs have gained ample attention in the recent past as deuteration of selected moieties in established medicaments can lead to improved pharmacokinetic properties.¹ Because C–D bonds (D = ²H) are more stable than C–H bonds with respect to enzymatic degradation, deuterated drugs feature a prolonged half-life time in the body,² which allows one to reduce their dosage and attenuate side effects.^{3,4} From an analytical viewpoint, deuteration of drugs offers novel possibilities for nuclear magnetic resonance (NMR) spectroscopy. NMR is a key method to investigate interactions of both drugs and target proteins. Complementary to methods such as isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR) that provide global thermodynamic and kinetic parameters about drug/target systems, solution-state NMR can localize drug binding sites and characterize their dynamics at atomic resolution. In addition, the affinity of drugs or their metabolites for macromolecules such as proteins⁵ can be studied by a wealth of NMR techniques that exploit, for example, contrasts in *T*₂ or *T*_{1ρ} as well as relaxation rates of long-lived states or long-lived coherences upon binding.^{6,7} Alternatively, differences in signal intensities can be observed upon selective saturation of one of the binding partners, known as saturation transfer difference (STD) spectroscopy,^{8,9} or of the solvent, known as water-LOGSY.^{10,11} In the past years, dissolution dynamic nuclear polarization (D-DNP) has also become increasingly popular as a tool for drug screening and ligand binding studies. For example, Lerche et al. have demonstrated how to use carbon-13 signal losses to identify the binding of small ligands to

macromolecular hosts;¹² Hilty and co-workers have shown how to employ relaxation dispersion techniques in combination with ¹³C and ¹⁹F detection,^{13,14} while Wilson and co-workers used changes in longitudinal ¹³C relaxation upon ligand binding in magnetic resonance imaging.¹⁵

To expand NMR and D-DNP to deuterated drugs and metabolites, we enlarge the NMR toolbox by monitoring effects on long-lived nuclear spin states (LLS) in deuterated methyl groups (CD₃). The relaxation rates *R*_{LLS} = 1/*T*_{LLS} of these LLS are sensitive to the frequency of internal CD₃ rotation, characterized by the frequency, thus enabling one to identify interactions between target proteins and drugs or metabolites containing deuterated methyl groups. LLS involving two or more deuterons have been described as imbalances between populations of nuclear spin states of different symmetry.¹⁶ The flow of populations between different symmetry manifolds is forbidden to first order, so that population imbalances have a prolonged lifetime compared to spin–lattice relaxation times.

Different kinds of LLS have been reported in CH₂ and CH₃ groups by Dumez, Levitt, Jannin, and co-workers^{17–19} and in CD₂ and CD₃ groups by Kurzbach et al.²⁰ and by Jhaharia et al.²¹ In deuterated methyl groups, LLS return to equilibrium populations with rates that depend on (i) the correlation time

τ_c of the overall rotational diffusion of the molecule (here assumed to be isotropic), (ii) the internal rotation of the methyl group around its three-fold symmetry axis with a correlation time $\tau_c \ll \tau_r$. Both τ_c and τ_r can be affected by the binding of a molecule comprising a deuterated CD_3 group to a target protein, hence revealing their mutual interaction.

To produce the LLS that serve here as sensors for host-guest interactions, we applied the D-DNP²² technique to deuterated dimethyl sulfoxide ($DMSO-d_6$) with carbon-13 in natural abundance, which we shall use as a model for drugs or metabolites containing deuterated methyl groups.

D-DNP aims at boosting NMR signal intensities by transferring the magnetization of unpaired electrons of paramagnetic agents (PAs) such as 1- λ^1 -oxidanyl-2,2,6,6-tetramethylpiperidin-4-ol (TEMPO) to the nuclear spins of a molecule of interest. Our D-DNP experiments proceeded in three steps: (i) Partial saturation of the electron spin resonance spectrum of samples containing 50 mM TEMPO using microwave irradiation ($\nu_{\mu w} = 187.9$ GHz, $B_{0,DNP} = 6.7$ T), which results in direct positive hyperpolarization of both ^{13}C and 2H nuclei. (The optimal microwave frequencies for these nuclei do not differ significantly under our experimental conditions.²³) Nuclear hyperpolarizations $P_1(^{13}C) > 15\%$ and $P_1(^2H) > 15\%$ can be achieved at cryogenic temperatures of $T_{DNP} = 1.2$ K. The deuterium hyperpolarization reaches a plateau after saturating the EPR transitions of TEMPO by microwave irradiation for ca. 1 h (see Figure S6, Supporting Information) with a characteristic time constant of ca. 18 min. (ii) After the polarization has built up, the frozen hyperpolarized sample is dissolved with 5 mL of overheated D_2O ($T_{D_2O} = 453$ K at $P_{D_2O} = 1.05$ MPa) and pushed within 3 s through a magnetic tunnel ($B_{0,tunnel} = 0.9$ T) to a solution-state NMR spectrometer, where a fraction of ca. 1 mL of the bolus is injected into 1 mL of a buffer solution with or without target proteins (Figure 1). (iii) For all experiments reported here, ^{13}C NMR spectra of hyperpolarized $DMSO-d_6$ are acquired at intervals of 0.5 s by applying 15° detection pulses (see Figure S1 in the SI). Spectra are acquired up to 80 s after injection, when the signals become too weak for detection.

The LLS were monitored indirectly via ^{13}C NMR of the multiplets of the CD_3 groups. In thermal equilibrium, the spectrum contains seven lines (see Figure 2) that feature a trinomial intensity distribution 1:3:6:7:6:3:1, which results from couplings $^1J(^{13}C, ^2H) = 21.2$ Hz to three magnetically equivalent deuterium spins with spin $I = 1$. After a DNP buildup time of >1 h without cross-polarization, the ^{13}C multiplets benefit from ca. 10 000-fold signal enhancements with respect to their amplitudes in thermal equilibrium (typically detected by averaging 1024 scans over a period of 96 h). The time dependence of the seven line intensities after injection allows one to determine the lifetime of the LLS. Figure 2 shows a hyperpolarized multiplet 3 s after injection (Figure 2a) and a partially relaxed spectrum 60 s after injection (Figure 2b). The population imbalance is evidenced, inter alia, through increased relative intensities of lines L_1 and L_7 and a reduced relative intensity of L_4 . Note that the multiplets are asymmetric about their centers (e.g., line L_1 is stronger than L_7 etc.), hinting at the presence of C_2D_z and $C_2D_zD_z'D_z''$ order (see Figure S4 in the SI).

Monitoring of LLS. The averaged ("lumped") populations¹² P_A , P_B , and P_E of the three manifolds of symmetry A, B, and E $= E_1 \oplus E_2$ can be monitored as a function of time after injection. The lifetime $T_{LLS}(^2H)$ was up to 6 times longer than

Figure 1. Hyperpolarized NMR can be exploited for the detection of LLS in ligands, drugs, or metabolites comprising deuterated CD_3 groups. A frozen solution (blue) of a ligand such as $DMSO-d_6$ containing 50 mM of the radical TEMPO is hyperpolarized by microwave irradiation (here at 187.9 GHz) in a dedicated DNP apparatus that operates at a temperature of 1.2 K and a magnetic field of 6.7 T. The sample is then dissolved with a burst of 5 mL of superheated D_2O at 10.5 bar and $180^\circ C$ (red box) and transferred within 3 s to an NMR spectrometer operating at 9.4 T (400 MHz for 1H) where a fraction is injected into an NMR tube that contains a buffered protein solution (green). Finally, a series of ^{13}C NMR spectra of the ligand $DMSO-d_6$ are acquired at regular intervals after injection.

$T_1(^2H)$ in $DMSO-d_6$ under our experimental conditions. Figure 2c shows the energy levels in a D_3 spin system with their assignment to different symmetry manifolds. The following normalized linear combinations of line intensities allow one to determine the lumped normalized populations¹⁶

$$\begin{aligned} P_A &= 5(L_1 + L_7)/L_{tot} \\ P_B &= \left[L_4 - \frac{1}{2}(L_3 + L_5) \right] / L_{tot} \\ P_E &= [2(L_3 + L_5) - 4L_1 - 4L_7] / L_{tot} \\ L_{tot} &= \sum_i L_i \end{aligned} \quad (1)$$

Figure 3 depicts the evolution of the lumped normalized populations P_A , P_B , and P_E after injection into the NMR spectrometer. They tend exponentially toward their thermal equilibrium distribution where all 27 states have approximately the same populations ($P_A^{eq} = 10/27 \simeq 0.37$; $P_E^{eq} = 16/27 \simeq 0.59$; $P_B^{eq} = 1/27 \simeq 0.037$). Evidently, the LLS corresponds to an overpopulation $P_A/P_A^{eq} > 1$ and a deficiency $P_E/P_E^{eq} < 1$, i.e., to a positive A/E spin-state imbalance with respect to thermal equilibrium, where $P_A^{eq}/P_E^{eq} = 10/16 = 0.625$. Once turbulences after injection into the NMR spectrometer have died out (see the SI), we find initial polarizations up to $P_A \approx 0.46$ or $P_A/P_A^{eq} \approx 1.24$ and $P_E \approx 0.51$ or $P_E/P_E^{eq} \approx 0.86$ and a ratio $P_A/P_E \approx 0.9$. By analogy to earlier work,²⁴ the polarization of the long-lived A/E imbalance can be defined as a ratio

$$P_{LLS}(^2H) = \left(\frac{P_A}{P_A^{eq}} - \frac{P_E}{P_E^{eq}} \right) / \left(\frac{P_A}{P_A^{eq}} + \frac{P_E}{P_E^{eq}} \right) \quad (2)$$

which in the case at hand (Figure 3) is estimated to be ca. 18%. The decay time constants of the imbalance $P_{LLS}(^2H)$ can be as long as $T_{LLS}(^2H) = 4.3$ s in protein-free, buffered solutions

Figure 2. Typical ^{13}C multiplets of the $^{13}\text{CD}_3$ groups in $\text{DMSO-}d_6$ (deuteration > 99.9% to reduce signals of partly protonated methyl groups) with ^{13}C in natural abundance obtained (a) 3 and (b) 60 s after injection into a buffer solution without any proteins. The LLS lead to deviations of the septet from the trinomial high-temperature intensity distribution 1:3:6:7:6:3:1, in particular, to attenuation of the inner line intensities L_3 , L_4 , L_5 , and L_6 compared to the outer lines L_1 and L_7 . The signals of the seven components are shown in light blue; their sums are represented by dashed blue lines. The fit with the experimental multiplets (orange lines) is satisfactory. (c) Energy-level diagram of a D_3 spin system with 27 states belonging to three different irreducible representations of the C_{3v} point group A, B, and E. By D-DNP with microwave irradiation of the electron spin transitions of TEMPOL radicals at 1.2 K, states of symmetry A can be overpopulated, while states of symmetry B and E are partly depleted, thus boosting lines L_1 and L_7 in the multiplet.¹⁶ The number of spin states is increased to 54 when the ^{13}C nucleus is taken into account.

Figure 3. Evolution of the normalized lumped populations P_A , P_B , and P_E determined from seven experimental line intensities L_i via eq 1 as a function of time after turbulences have died out (see the SI; $t = 0$ s in the figure corresponds to 2 s after injection). The fitted time constant of the decay of the A/E imbalance is $T_{\text{LLS}}(^2\text{H}) = 4.6 \pm 0.2$ s. The vertical scale was calibrated by normalizing the total polarization to unity ($P_A + P_B + P_E = 1$).

Figure 4. Simulated time traces of the averaged (lumped) populations P_A (red), P_E (green), and P_B (blue) in a D_3 spin system for $\langle\omega_Q\rangle/(2\pi) = 67$ kHz, $\tau_c = 100$ ps, and (a) $\tau_r = 1$ ps and (b) $\tau_r = 2.5$ ps, starting from a hypothetical “perfect” initial imbalance $P_A = 1$; $P_B = P_E = 0$. In our experiments, the initial conditions are $P_A < 1$, $P_B > 0$, and $P_E > 0$. The rates R_{LLS} are 0.26 and 0.64 s^{-1} in (a) and (b), respectively. Note that the values of τ_c and τ_r used for the simulations serve only for demonstration and should be considered with caution. The fitting of complex relaxation equations to experimental time traces is largely underdetermined for the case at hand (see ref 26).

The simplification of eq 4 is allowed assuming that $0.1 < \tau_c < 178$ 1 ns for $\text{DMSO-}d_6$ in complexes with macromolecular partners, 179 while it is $0.1 < \tau_r < 10$ ps for methyl group rotation in DMSO- 180 d_6 , so that the inequality $\tau_r \ll \tau_c$ is fulfilled by at least 1 order 181 of magnitude. Thus, the rate R_{LLS} in eq 4 is directly 182 proportional to the correlation time of the internal methyl 183 group rotation. For example, if $\tau_c = 0.1$ ns and $\langle\omega_Q\rangle/(2\pi) = 67$ 184 kHz, an increase in τ_r from 1 to 2.5 ps would entail a drop in 185 $T_{\text{LLS}}(^2\text{H})$ from 3.8 to 1.6 s that should be readily observable in 186 our experiments. LLS relaxation times $1 < T_{\text{LLS}}(^2\text{H}) < 30$ s lie 187

$$R_{\text{LLS}} = \frac{15}{4} \frac{\tau_c \tau_r (2\tau_c + \tau_r) \langle\omega_Q\rangle^2}{(\tau_c + \tau_r)(4\tau_c + \tau_r)} \quad (3)$$

$$\sim \frac{15}{8} \tau_r \langle\omega_Q\rangle^2 \quad \text{for } \tau_r \ll \tau_c \quad (4)$$

Figure 5. Decay of the lumped populations P_A (red), P_E (green), and P_B (blue) of the three symmetry manifolds upon injection into (a) neat buffer, (b) BSA, and (c) trypsin solutions. The return of the populations to equilibrium is the slowest, i.e., the lifetime $T_{LLS}(^2H)$ is the longest, in the absence of any protein and shorter in the presence of BSA than in solutions containing trypsin, indicating a stronger affinity and a more restrained internal methyl group rotation in the former case. The vertical scale was calibrated by normalizing the total polarization to unity ($P_A + P_B + P_E = 1$). The TEMPOL concentrations after injection were comparable in all three experiments, as evidenced by comparable $T_1(^{13}C)$, (a) 16.6 ± 0.1 , (b) 16.4 ± 0.1 , and (c) 14.7 ± 0.1 s, so that it is legitimate to compare the three experiments (see the main text).

in an appropriate range for detection by ^{13}C NMR, bearing in mind that very fast processes with $T_{LLS}(^2H) < 1$ s cannot be detected by current D-DNP technology due to the relatively slow transfer from the polarizer to the NMR system, and very slow processes with $T_{LLS}(^2H) > 30$ s would be masked by longitudinal ^{13}C relaxation, with lifetimes that are typically on the order of $T_1(^{13}C) = 30$ s in DMSO- d_6 in the absence of radicals.

Note that, provided $\tau_c \gg \tau_r$, longitudinal relaxation times $T_1(^2H)$ and $T_1(^{13}C)$ in methyl groups primarily depend on τ_c rather than on τ_r , while the opposite situation arises for $T_{LLS}(^2H)$ which depends mostly on τ_r and barely on τ_c . Hence $T_{LLS}(^2H)$ can report on small variations of τ_r caused by weak interactions with macromolecular partners that lead to some steric hindrance to rotation. Furthermore, it follows from eq 4 that $T_{LLS}(^2H)$ is largely independent of the mass of the complex formed with the macromolecular partner but depends on steric constraints that slow down the internal methyl group rotation.

Because quadrupolar relaxation is not very effective in diminishing an A/E imbalance, as evidenced by the long lifetimes $T_{LLS}(^2H)$, one may wonder if other relaxation mechanisms might become important. Dipolar 2H -relaxation is subject to similar symmetry constraints as quadrupolar relaxation, i.e., a flow of polarization between spin manifolds that belong to different irreducible representations within the C_{3v} point group through dipolar relaxation is also forbidden by first order, as discussed by Dumez et al.²⁶ and Ivanov et al.¹⁶ However, dipolar couplings between deuterons are over 1 order of magnitude weaker than quadrupolar couplings. Numerical simulations show that the effect of dipolar relaxation on an A/E imbalance is negligible compared to quadrupolar relaxation (see the SI). However, paramagnetic relaxation due to radicals such as TEMPOL significantly affects both $T_{LLS}(^2H)$ and $T(^{13}C)$, as shown in Figure 6.

Monitoring Ligand–Protein Interactions. The dependence of $T_{LLS}(^2H)$ on τ_r described by eq 4 can be put to use to identify ligand–protein interactions that are likely to perturb the methyl group rotation. This may be of interest for small molecules or ligands that are being considered as potential drugs. We compared interactions of DMSO- d_6 with bovine serum albumin (BSA) and trypsin from porcine pancreas. BSA is a transporter protein with binding pockets that can

accommodate small ligands such as DMSO, as evidenced by the effect of DMSO on PCR amplification.²⁷ In contrast, DMSO- d_6 is expected to interact less efficiently with trypsin, which is not a transporter protein. Hence, deuterated methyl groups in DMSO- d_6 are expected to be less perturbed by trypsin than by BSA, and the LLS are expected to live longer in the former case.

All $T_{LLS}(^2H)$ experiments were carried out in 10 mM phosphate buffer saline (PBS) adjusted to pH 7.4. The final protein concentrations were 225 ± 45 μ M, and the final ligand concentrations were 350 ± 150 mM depending on the injection (see the SI). Figure 5 shows that the lifetimes can be ranked in the sequence $T_{LLS}(\text{protein-free}) = 3.2 \pm 0.6$ s $>$ $T_{LLS}(\text{trypsin}) = 1.6 \pm 0.2$ s $>$ $T_{LLS}(\text{BSA}) = 1.4 \pm 0.1$ s. As expected, the decay is faster with BSA than that with trypsin. When considering the fast equilibrium between free and bound DMSO and the interactions on the surface or in the binding pockets, the methyl group rotation appears to be more constrained in the DMSO/BSA complex than that in the DMSO/trypsin case.

The ligand–protein interactions were studied under similar experimental conditions by ITC: flat ITC profiles (see Figure S2 in the SI) indicate that the interactions between DMSO- d_6 and BSA are nonspecific, with an interaction heat $\Delta H = +142.6 \pm 14.6$ J/mol at 298 K, while interactions between DMSO- d_6 and trypsin yield $\Delta H = +119.3 \pm 6.7$ J/mol. This points toward slightly stronger nonspecific binding of DMSO- d_6 to BSA than to trypsin, presumably because BSA offers amphiphilic binding pockets.²⁸

Reproducibility. Because of shortcomings of current instrumentation, D-DNP experiments can be subject to significant uncertainties, in particular, when the volume of the injected solutions and the final concentrations of the hyperpolarized molecules and radicals are not perfectly controlled. In most D-DNP studies that have been carried out so far, such variations had little effect on the outcome of the experiments. However, in comparisons such as those illustrated in Figure 5, one must pay attention to various details. First, the reproducibility of the dissolution process is necessarily limited because the frozen glassy sample that was hyperpolarized at 1.2 K must be dissolved by a burst of superheated solvent. The efficiency of this process depends (i) on the speed of the dissolution (which determines the fraction

of surviving hyperpolarization), which in turn depends on the volume and morphology of the frozen sample (e.g., a solid block, small granules, etc.) and (ii) on the fraction of the heat capacity of the superheated solvent that is wasted upon warming up the probe and cryostat. As a consequence of these factors, the bolus of the hyperpolarized liquid that travels from the polarizer to the NMR spectrometer before injection may feature a varying concentration gradient, which affects the final concentration if only 20% (here, the leading 1 mL fraction out of 5 mL) of the bolus is injected (see the SI for details). Such factors influence the final ligand and TEMPOL concentrations, which, if not appropriately controlled, can lead to significant variations in the observed relaxation rates.

Figure 6 shows empirical correlations of $T_{LLS}(^2H)$ and $T_1(^{13}C)$ for 4–6 independent repetitions of experiments on

Figure 6. Correlations of lifetimes $T_{LLS}(^2H)$ vs $T_1(^{13}C)$ in DMSO- d_6 measured in six independent D-DNP experiments without target protein (green dots), four experiments in the presence of trypsin (red crosses), and four experiments in the presence of BSA (blue diamonds). Variations in TEMPOL concentrations upon injection (see main text) affect both $T_{LLS}(^2H)$ and $T_1(^{13}C)$ by paramagnetic relaxation enhancement. The dashed lines indicate the slopes $\alpha = \Delta T_{LLS}(^2H) / \Delta T_1(^{13}C)$ for the three different cases (no protein, trypsin, or BSA). Variations of the longitudinal relaxation times $T_1(^{13}C)$ reflect variations of the TEMPOL concentration, while deuterium relaxation $T_1(^2H)$ is not significantly affected (see the SI).

three different samples (altogether 14 independent runs): protein-free, with BSA and with trypsin. Evidently, $T_{LLS}(^2H)$ and $T_1(^{13}C)$ vary significantly in all three cases. Even in the absence of any target proteins, variations of $12 < T_1(^{13}C) < 18$ s were observed. Although not observed, slight fluctuations in sample temperature after dissolution and mixing cannot be ruled out completely. However, the considerable variations of $T_{LLS}(^2H)$ and $T_1(^{13}C)$ cannot be attributed to variations in correlation times τ_c of overall rotational diffusion as a consequence of temperature fluctuations. Furthermore, control experiments without radicals indicated that $T_1(^{13}C)$ of DMSO- d_6 is not significantly affected by the presence of the target proteins: $T_1(^{13}C) = 33.7 \pm 5.2$ s in the absence of any protein and $T_1(^{13}C) = 30.9 \pm 4.7$ s in the presence of BSA. Thus, the wide range $10 < T_1(^{13}C) < 30$ s observed in Figure 6 can only be ascribed to varying concentrations of paramagnetic species. As shown in Figure S5 in the SI, the value of $T_1(^{13}C)$ can provide a measure of the TEMPOL concentration. Variations of $12 < T_1(^{13}C) < 30$ s correspond to $0.08 > R_1(^{13}C) > 0.033$ s $^{-1}$ that translates into an estimation of the TEMPOL concentrations in a range between ca. 1 and 4 mM (see Figure S5 in the SI). At least three methods could help to overcome the problem of variable radical concentrations:

filtration of radicals attached to surfaces of porous solids,²⁹ scavenging of the radicals by chemical reduction,³⁰ or a rapid transfer of hyperpolarized solid samples combined with subsequent dissolution near the NMR spectrometer.³¹

The parameters $T_{LLS}(^2H)$ and $T_1(^{13}C)$ in Figure 6 are roughly linearly correlated for the three mixtures (protein-free, BSA, or trypsin), which is in line with the linear dependence of slow relaxation rates on radical concentrations (“relaxivity”). The slopes of $T_{LLS}(^2H)$ vs $T_1(^{13}C)$ are distinct for the three cases. Indeed, the slope is largest in the absence of any protein, corroborating our finding that $T_{LLS}(^2H)$ tends to be longer in the absence of proteins.

Clearly, improved control over the radical concentration would greatly facilitate the quantitative use of D-DNP for interaction studies. To improve the reliability of D-DNP, $T_{LLS}(^2H)$ times should ideally be compared with similar TEMPOL concentrations (as in Figure 5) or by considering characteristic slopes $\alpha = \Delta T_{LLS}(^2H) / \Delta T_1(^{13}C)$ in correlation plots such as in Figure 6 or, if possible, by taking precautions to remove the radicals.

In conclusion, the lifetime $T_{LLS}(^2H)$ of the A/E imbalance in deuterated methyl groups is longest in the absence of any protein as the methyl group rotation is not perturbed. The interactions between DMSO- d_6 and BSA or trypsin slow down the methyl group rotation, leading to a shortened $T_{LLS}(^2H)$. Hence, the lifetime $T_{LLS}(^2H)$ is a good indicator of weak interactions of DMSO- d_6 with macromolecules such as proteins. The method can be generalized to other ligands with deuterated methyl groups and may offer a novel approach to study weak affinities of deuterated drugs where other techniques might lack sensitivity. Furthermore, it can provide information about a complex’s morphology as the internal methyl group dynamics will only be perturbed if the group takes part in the formation of the interaction interface.

LLS in deuterated methyl groups represent a complementary approach to other, more or less established, drug-screening techniques such as STD spectroscopy,³² Water-LOGSY,¹¹ contrast of transverse relaxation rates R_2 or $R_{1\rho}$ due to binding,^{6,33} contrast of relaxation rates R_{LLC} of long-lived coherences,³⁴ or contrast of relaxation rates R_{LLS} of long-lived states.⁷ Such techniques usually rely on the detection of nuclei with high gyromagnetic ratios, such as 1H or ^{19}F , because detection of ^{13}C nuclei in natural abundance often does not provide sufficient sensitivity for ligand titration experiments. Hyperpolarization of ^{13}C nuclei can overcome this hurdle, and the indirect detection via ^{13}C of the lifetimes $T_{LLS}(^2H)$ in hyperpolarized methyl groups can provide an attractive means to characterize weak interactions.

Because the dispersion of chemical shifts is more favorable for ^{13}C than that for 1H , the method presented here allows one to study a mixture of ligands in a single experiment, provided that the ^{13}C multiplets of the methyl groups of different ligands do not overlap. It remains to be seen to what extent the methodology presented here will be complementary to 1H -based STD, Water-LOGSY, and R_2 or $R_{1\rho}$ studies at millimolar concentrations.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.9b00149.

Experimental details of D-DNP; NMR observation after dissolution and injection; isothermal titration calorimetry (ITC); simulations of the spin population dynamics; observation of antiphase terms; paramagnetic contributions to longitudinal carbon-13 relaxation; and buildup of deuterium polarization by DNP ([PDF](#))

AUTHOR INFORMATION

Corresponding Authors

*E-mail: dennis.kurzbach@univie.ac.at.

*E-mail: geoffrey.bodenhausen@ens.fr.

ORCID

Thomas Kress: 0000-0001-9133-4310

Astrid Walrant: 0000-0003-4054-905X

Dennis Kurzbach: 0000-0001-6455-2136

Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Prof. Hans Dahn on the occasion of his 100th birthday.

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