Supplementary Materials for

Long-lived states of methylene protons in achiral molecules

Anna Sonnefeld et al.

Corresponding author: Kirill Sheberstov, kirill.sheberstov@ens.psl.eu

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Supplementary Text

Analytical treatmen
t of LLS excitation by single SLIC in AA’XX’ systems

The $J$-coupling Hamiltonian of a four-spin $AA’XX’$ spin system can be written as:

$$
\hat{H}_J = J_{AA'} I_A^A \cdot I_A^{A'} + J_{XX'} I_X^X \cdot I_X^{X'} + \frac{1}{2} \sum (I_A^A + I_A^{A'}) (I_X^X + I_X^{X'}) + \frac{1}{2} \Delta J (I_A^A - I_A^{A'}) (I_X^X - I_X^{X'}).$$

(S1)

where the $J_{AA'}$ and $J_{XX'}$ are the geminal $J$-couplings, while the parameters $\Sigma J = 3 J_{AX} + 3 J_{AX'}$ and $\Delta J = 3 J_{AX} - 3 J_{AX'}$ represent the sum and difference of the vicinal $J$-couplings. During monochromatic spin-lock induced crossing (single SLIC), a single radio frequency (RF) pulse is applied on resonance with the two spins $A$ and $A'$ (or, mutatis mutandis, with the two spins $X$ and $X'$):

$$
\hat{R}_{RF} = -\nu_{RF} (I_A^A + I_A^{A'}).$$

(S2)

A convenient basis to describe the spin dynamics in the manifold of $2^4 = 16$ states of this spin system is obtained by taking all direct products of the 4 states of spins $A$ and $A'$ (three triplet states $T^m_{AA'}$ with the quantum numbers $m = \{+1, 0, -1\}$, and the singlet state $S^A_{AA'}$) with the corresponding 4 states of spins $X$ and $X'$. When a monochromatic SLIC pulse is applied along the $x$ axis of the rotating frame, it is convenient to consider the triplet states tilted by 90°, as proposed by DeVience et al. (25, 42). The triplet states in the tilted frame result from the transformation:

$$
\tilde{T}^m_{AA'} = (\tilde{R}_x^A (\pi/2) + \tilde{R}_y^A (\pi/2)) T^m_{AA'},
$$

(S3)

where the operator $\tilde{R}_y^A (\pi/2) = \exp (-i \frac{\pi}{2} I_y^A)$ describes a rotation of spin $I = A$ or $A'$ around the $y$-axis through an angle of $\pi/2$. The singlet states $S^A_{AA'}$ and $S^X_{XX'}$ and the triplet states of non-irradiated spins $T^m_{XX'}$ remain invariant. The resulting 16 product states are then sorted according to their symmetry (gerade or ungerade) with respect to intrapair permutations of spins AA’ and XX’. We shall adopt the same ordering of states in the rotating frame as proposed by Stevanato et al. (64):
\[
{S^4 \mathcal{T}^4 S^x T^x} = \begin{pmatrix}
\mathcal{T}_{A^4}^0 T_{x^4}^0, \\
\mathcal{T}_{A^4}^1 T_{x^4}^0, \\
\mathcal{T}_{A^4}^0 T_{x^4}^1, \\
\mathcal{T}_{A^4}^1 T_{x^4}^1, \\
\mathcal{T}_{A^4}^0 T_{x^4}^0, \\
\mathcal{T}_{A^4}^1 T_{x^4}^0, \\
\mathcal{T}_{A^4}^0 T_{x^4}^1, \\
\mathcal{T}_{A^4}^1 T_{x^4}^1, \\
\mathcal{T}_{A^4}^0 T_{x^4}^0 \\
\end{pmatrix}
\]

The first 9 states correspond to triplet-triplet product states, the 10th state (highlighted in bold) is the ‘pure’ singlet-singlet product state, followed by three ‘mixed’ triplet-singlet product states and three ‘mixed’ singlet-triplet product states.

The dynamics of the excitation of LLS is governed by the sum of the Hamiltonians \( \hat{H}_{SLIC} = \hat{H}_R + \hat{H}_J \), given in Eq. (S1) and Eq. (S2). In the rotating-frame basis of Eq. (S4), \( \hat{H}_{SLIC} \) has a block-diagonal form. For methylene groups, the geminal intrapair couplings are usually similar \( J_{AA'} \approx J_{XX'} \), so that only the block containing the first 10 basis functions (the 9 triplet-triplet states \( S_0^{AA'} S_0^{xx'} \) and the singlet-singlet state \( S_0^{AA'} S_0^{xx'} \)) is important for SLIC. This gerade 10 x 10 block has the following form:

\[
\begin{pmatrix}
A - \nu_{RF} & -C & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-C & A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A - \nu_{RF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A - \nu_{RF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A + \nu_{RF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & A + \nu_{RF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -A & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -A & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{pmatrix}
\]

where \( A = \frac{1}{4} (J_{AA'} + J_{XX'}) \approx \frac{1}{2} J_{AA'} \), \( B = -\frac{3}{4} (J_{AA'} + J_{XX'}) \approx -\frac{3}{2} J_{AA'} \), \( C = \frac{\sqrt{2}}{4} \sigma J = \frac{\sqrt{2}}{4} (3J_{AX} + 3J_{AX'}) \), \( D = \frac{\sqrt{2}}{4} \Delta J = \frac{\sqrt{2}}{4} (3J_{AX} - 3J_{AX'}) \).

Seven of the nine triplet-triplet states are disconnected from the remaining states, but the two states \( T_{A^4}^0 T_{x^4}^0 \) and \( T_{A^4}^0 T_{x^4}^0 \) are connected with \( S_0^{AA'} S_0^{xx'} \) through two off-diagonal elements \( \pm D = \pm \frac{\sqrt{2}}{4} \Delta J \) highlighted in red. The ungerade 6x6 block does not play a role for methylene groups because the differences between the diagonal elements almost vanish, so that the singlet and triplet states are strongly mixed and are not accessible for SLIC. In general, there may exist spin systems...
where either $J_{AA'}$ or $J_{XX'}$ almost vanishes, so that SLIC can create LLS imbalances within both the gerade and ungerade manifold at the same time.

Typical magnitudes of the matrix elements can be obtained from experimental values for a selection of molecules, that are summarized in Table 1 in the main text. The difference between the diagonal elements $A - B = J_{AA'} + J_{XX'} \approx 2J_{AA'}$ lies in range between 25 to 29 Hz, while the sum of the vicinal $J$-couplings varies in the range $10 \leq \Sigma J \leq 14$ Hz. Most importantly, the crucial difference $\Delta J$ between the vicinal $J$-couplings lies in the range $0 \leq \Delta J \leq 6.5$ Hz.

The first 90° pulse in SLIC pulse sequence (see Fig. 4a in the main text) leads to an overpopulation of the $\tilde{T}^{AA'}_{+1} T^{XX'}_0$ state and a depopulation the $\tilde{T}^{AA'}_{-1} T^{XX'}_0$ state with respect to the initial (thermal) Boltzmann distribution of populations. When the nutation (Rabi) frequency $\nu_{RF}$ is chosen to match the difference between the diagonal elements $\nu_{RF} = A - B = J_{AA'} + J_{XX'} \approx 2J_{AA'}$, the $\tilde{T}^{AA'}_{+1} T^{XX'}_0$ state becomes degenerate with the $S^{AA'}_0 S^{XX'}_0$ state so that the difference of the populations of the these two states starts to oscillate with a frequency determined by off-diagonal element $D = \sqrt{2}/4 \Delta J$. After RF irradiation of duration $\tau_{SLIC} = 1/(\sqrt{2} \cdot |\Delta J|)$ (or an odd multiple thereof), the populations are completely exchanged. If, on the other hand, $\nu_{RF}$ is chosen to match the difference between the diagonal elements $B - A = -(J_{AA'} + J_{XX'}) \approx -2J_{AA'}$, a similar situation arises between the $\tilde{T}^{AA'}_{-1} T^{XX'}_0$ and $S^{AA'}_0 S^{XX'}_0$ states. Therefore, by reversing the phase of the SLIC pulse from $+\nu_{RF}$ to $-\nu_{RF}$ (or, equivalently, by reversing the phase of the initial 90° pulse that precedes the SLIC pulse), it is possible to either overpopulate or depopulate $S^{AA'}_0 S^{XX'}_0$ state. This illustrates how phase cycling results in “singlet order selection” (SOS) (18).

Analytical treatment of LLS excitation by double SLIC in AA’XX’ systems

A similar approach can be used to treat the spin dynamics occurring during double SLIC. In this case, all four spins are irradiated simultaneously using two selective RF fields centred at the chemical shifts of the A and X nuclei:

$$\tilde{H}_{RF} = -\nu_{RF} \left( \hat{i}_A^x + \hat{i}_A' + \hat{i}_X^x + \hat{i}_X' \right).$$ (S6)

This Hamiltonian is written in the doubly rotated frame. A different basis is appropriate in this case because double RF irradiation leads to additional symmetries on the eigenstates. First, the triplet states of both AA’ and XX’ spin pairs should be rotated from the laboratory-frame $S^A T^A S^X T^X$ states into the rotating frame basis $S^A \tilde{T}^A S^X \tilde{T}^X$ by applying two $\tilde{R}_Y^{\pi/2}$ and $\tilde{R}_Y^{\pi/2}$ rotations, in addition to those of Eq. (S3). A suitable basis can be derived by using the theorems of the addition of angular momenta. The terms representing spins A and A’ can be coupled with those of spins X and X’ to produce a new set of states that are characterised by the total spin $F_A$ of the of AA’ pair, the total spin $F_X$ of the XX’ pair, and the total spin $F$ of the full 4-spin system, and the projection quantum number $m$. Each of the 16 symmetrized states is then uniquely defined by 4 quantum numbers $|F_A, F_X, F, m\rangle$. The states $\{\tilde{T}^{AA'}_{+1}, \tilde{T}^{AA'}_{0}, \tilde{T}^{AA'}_{-1}, S^{AA'}_0\}$ for the AA’ pair and $\{\tilde{T}^{XX'}_{+1}, \tilde{T}^{XX'}_{0}, \tilde{T}^{XX'}_{-1}, S^{XX'}_0\}$ for the XX’ pair can be represented in terms of the total spin $F$ and its projection $m$, i.e., $\tilde{T}^{AA'}_{+1} = |F_A = 1, m_A = 1\rangle$, $S^{AA'}_0 = |F_A = 0, m_A = 0\rangle$, and $\tilde{T}^{XX'}_{+1} = |F_X = 1, m_X = 1\rangle$, $S^{XX'}_0 = |F_X = 0, m_X = 0\rangle$. The symmetrized states can be expressed in terms of product states as follows:
\[ |F_A, F_X, m⟩ = \sum_{{m_A = -F_A}}^{F_A} \sum_{{m_X = -F_X}}^{F_X} C^{F,m}_{{F_A, m_A, F_X, m_X}} |F_A, m_A; F_X, m_X⟩, \]  

(S7)

with the Clebsch-Gordan coefficients \( C^{F,m}_{{F_A, m_A, F_X, m_X}} \) defined by:

\[ C^{F,m}_{{F_A, m_A, F_X, m_X}} = (F_A, m_A; F_X, m_X|F, m), \]  

(S8)

The analytical values of the Clebsch-Gordan coefficients can be calculated using recursive expressions and are listed in various textbooks and in software packages such as Wolfram Mathematica. Equation (S7) can be used to generate all possible combinations of the total spin \( F \) for different combinations of \( F_A \in \{0, 1\} \) and \( F_X \in \{0, 1\} \):

\[ |F_A - F_X| \leq F \leq F_A + F_X. \]  

(S9)

As result of the addition of angular momenta, the symmetrised 16-dimensional basis of the AA'XX' four-spin system contains several manifolds: 5 states with \( |F_A = 1, F_X = 1, F = 2⟩ \), 1 state with \( |F_A = 1, F_X = 1, F = 0⟩ \), 1 state with \( |F_A = 0, F_X = 0, F = 0⟩ \), 3 states with \( |F_A = 1, F_X = 1, F = 1⟩ \), 3 states with \( |F_A = 0, F_X = 1, F = 1⟩ \), and 3 states with \( |F_A = 1, F_X = 0, F = 1⟩ \). We used the Spin Dynamica software (65) to calculate the Hamiltonian in this symmetrized basis, where the Hamiltonian appears in factorised form, thus allowing a straightforward analysis of the spin dynamics. The block that is relevant for the LLS is spanned by 7 states that are disconnected from all 9 remaining states; this block comprises 5 triplet states with total spin \( F = 2 \) and \( m = +2, +1, 0, -1, -2 \), the state with \( |F_A = 1, F_X = 1, F = 0⟩ \), and the unique singlet-singlet state \( |F_A = 0, F_X = 0, F = 0⟩ \):

\[
\begin{bmatrix}
A - 2\nu_{RF} & 0 & \frac{1}{2\sqrt{6}} \Sigma J & 0 & 0 & -\frac{1}{4\sqrt{3}} \Sigma J & \frac{1}{4} \Delta J \\
0 & A - \nu_{RF} + \frac{1}{4} \Sigma J & \frac{1}{4} \Sigma J & 0 & 0 & 0 & 0 \\
\frac{1}{2\sqrt{6}} \Sigma J & 0 & A + \frac{1}{3} \Sigma J & 0 & \frac{1}{2\sqrt{6}} \Sigma J & \frac{1}{6\sqrt{2}} \Sigma J & -\frac{1}{2\sqrt{6}} \Delta J \\
0 & \frac{1}{4} \Sigma J & 0 & A + \nu_{RF} + \frac{1}{4} \Sigma J & 0 & 0 & 0 \\
0 & 0 & \frac{1}{2\sqrt{6}} \Sigma J & 0 & A + 2\nu_{RF} - \frac{1}{4\sqrt{3}} \Sigma J & \frac{1}{4} \Delta J \\
-\frac{1}{4\sqrt{6}} \Sigma J & 0 & \frac{1}{6\sqrt{2}} \Sigma J & 0 & -\frac{1}{4\sqrt{3}} \Sigma J & A - \frac{1}{3} \Sigma J & \frac{1}{2\sqrt{3}} \Sigma J \\
\frac{1}{4} \Delta J & 0 & -\frac{1}{2\sqrt{6}} \Delta J & 0 & \frac{1}{4} \Delta J & \frac{1}{2\sqrt{3}} \Sigma J & B
\end{bmatrix}
\]  

(S10)

Like in Eq. (S5), we have \( A = \frac{1}{4}(J_{AA'} + J_{XX'}) \approx \frac{1}{2}J_{AA'} \), and \( B = -\frac{3}{4}(J_{AA'} + J_{XX'}) \approx \frac{3}{2}J_{AA'} \), and the parameters \( \Sigma J = 3J_{AX} + 3J_{AX} \), and \( \Delta J = 3J_{AX} - 3J_{AX} \), represent the sum and difference of the vicinal \( J \)-couplings.

The diagonal and off-diagonal elements in (S10) that are important for the excitation of LLS are highlighted in red. When the nutation frequency \( \nu_{RF} \) of the double SLIC irradiation field matches \( \frac{1}{2}(J_{AA'} + J_{XX'}) \), either the \( |F_A = 1, F_X = 1, F = 2, m = 2⟩ \) state or the \( |F_A = 1, F_X = 1, F = 2, m = -2⟩ \) state becomes degenerate with the singlet-singlet state \( |F_A = 0, F_X = 0, F = 0, m = 0⟩ \). Like in single SLIC, the differences between the populations of these states starts to oscillate with a frequency given by off-diagonal element, which in this case has value of \( 1/4 \Delta J \). As a result, the optimum duration \( \tau_{\text{double-SLIC}} \) is longer than the duration \( \tau_{\text{single-SLIC}} \) by a factor \( \sqrt{2} \).
Fig. S1 shows energy level diagrams of an AA’XX’ system as a function of the nutation frequency $\nu_{RF}$ under single- and double-SLIC irradiation. With respect to single-SLIC, double-SLIC allows one to double the intensity of the observable signals after reconversion from LLS, since the population imbalances between states with $F = 2, m = \pm 2$ under double-SLIC are twice as large as between states with $F = 1, m = \pm 1$ under single SLIC. As discussed in the main text, double-SLIC allows one to address a chosen molecule in a highly specific manner, even if there is some signal overlap between CH$_2$ groups of several molecules. The drawback of double-SLIC is that the range of nutation frequencies $\nu_{RF}$ where level anti-crossings (LAC) occur is narrower than for single SLIC. In practice, this means that if $\Delta J < 1$ Hz, single SLIC may become more efficient than double SLIC, because the former is less sensitive to $B_1$ inhomogeneity. This observation is supported by numerical simulations as well as by experiments on $\beta$-mercaptoethanol (compound XI), where $\Delta J \approx 0.8$ Hz. In this case, the optimum signal amplitude derived from LLS by double-SLIC was about 90% of the signal intensity obtained with single SLIC, rather than 200% as expected. This also shows that the empirical adjustment of the RF amplitude is critical for double SLIC, especially when $\Delta J$ is small.

Yield of excitation and reconversion of LLS in AA’XX’ systems

The analysis of the spin dynamics during the single- and double-SLIC methods described in the previous sections reveals that the LLS excited corresponds to the population imbalance between the averaged population of the 9 triplet-triplet product states ($T^A_{m} T^{\prime X}_{n}$, $m, n \in \{+1, 0, -1\}$) and

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**Fig. S1.** Energy level diagrams for single and double SLIC. Energy levels of an AA’XX’ system as a function of the amplitude (nutation frequency) $\nu_{RF}$ of the RF pulse(s) during (a) single SLIC, and (b) double-SLIC, obtained by numerical diagonalization of the Hamiltonian $\hat{H}_{RF} + \hat{H}_J$. (a) Energies of the 9 triplet-triplet states $T^A_{k} T^{\prime X}_{l}$ and the unique singlet-singlet state $S^A_{0} S^{\prime X}_{0}$ (labelled $|0,0\rangle$.) (b) Energies of the 5 states with $|F_A = 1, F_X = 1, F = 2, m = \pm 2, -1, 0, +1, +2\rangle$, 1 state with $|F_A = 1, F_X = 1, F = 0\rangle$, one singlet-singlet state with $|F_A = 0, F_X = 0, F = 0\rangle$ (also labelled $|0,0\rangle$.) In both cases the following parameters were used for simulation: $J_{AA'} = -13$ Hz, $J_{XX'} = -14$ Hz, $\Delta J = 5.5$ Hz, $\Sigma J = 13$ Hz. Note that the vertical range in (b) is twice as large as in (a), while the horizontal range of the nutation frequency $\nu_{RF}$ is same in both graphs.
the unique singlet-singlet product state \( S_0^{AA'} S_0^{XX'} \). This imbalance can be represented as a linear combination of Cartesian product operators comprising two 2-spin products and one 4-spin product, as can be verified by inspection of the matrix representations:

\[
\sigma_{LS}^{AA'XX'} = -\frac{1}{3} (I^A \cdot I^{A'} + \mathbf{1}^X \cdot \mathbf{1}^{X'}) + \frac{8}{9} (I^A \cdot I^{A'}) (\mathbf{1}^X \cdot \mathbf{1}^{X'}). \tag{S11}
\]

In contrast to the 6-spin case treated in ref. (45), the coefficients \( \lambda = -1/3 \) and \( 8/9 \) are constrained by spin dynamics. It is straightforward to construct a matrix representation of this superposition of Cartesian product operators in the \( \{S^A T^A S^X T^X\} \) basis in the laboratory frame:

\[
\{S^A T^A S^X T^X\} = \begin{cases}
T_{+1}^{AA'} T_{+1}^{XX'}, \\
T_{-1}^{AA'} T_{+1}^{XX'}, \\
T_{0}^{AA'} T_{0}^{XX'}, \\
T_{+1}^{AA'} T_{-1}^{XX'}, \\
T_{0}^{AA'} T_{-1}^{XX'}, \\
T_{-1}^{AA'} T_{+1}^{XX'}, \\
T_{0}^{AA'} T_{0}^{XX'}, \\
T_{-1}^{AA'} T_{-1}^{XX'}, \\
S_{0}^{AA'} S_{0}^{XX'}, \\
T_{+1}^{AA'} S_{0}^{XX'}, \\
T_{0}^{AA'} S_{0}^{XX'}, \\
T_{-1}^{AA'} S_{0}^{XX'}, \\
S_{0}^{AA'} S_{0}^{XX'}, \\
S_{0}^{AA'} S_{0}^{XX'}, \\
S_{0}^{AA'} S_{0}^{XX'}, \
\end{cases}
\]

This yields the following matrix representation:

\[
\sigma_{LS}^{AA'XX'} = \frac{1}{9} \begin{pmatrix}
\begin{array}{cccccccccccc}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 9 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{array}
\end{pmatrix}
\]

This matrix indeed describes a population imbalance between the averaged population of the 9 triplet-triplet product states \( (T_n^{AA'} T_n^{XX'} \text{, } m, n \in \{+1, 0, -1\}) \), each of which has a population of \(-1/9\), and the singlet-singlet product state \( S_0^{AA'} S_0^{XX'} \) with a population of \(+1\), highlighted in red.
The theory of the excitation of LLS presented here only considers coherent contributions to the spin dynamics. The analysis of the relaxation superoperator in multiple-spin systems such as AA’XX’ systems shows that the state given in eq. (S11) is expected to have a long lifetime (49). However, barring detailed knowledge of all dipole-dipole interactions and their time-dependent fluctuations, the lifetime cannot be predicted.

Let us now consider the maximum expected intensities of LLS signal that can be obtained by single and double SLIC experiments. The initial thermal equilibrium is described by the following state:

$$\sigma_{\text{thermal}} = \frac{1}{16} \mathbb{1} + \frac{p_H}{16} \left( \hat{i}_z^A + \hat{i}_z^{A'} + \hat{i}_x^A + \hat{i}_x^{A'} \right), \text{with } p_H = \frac{\gamma_H B_0 h}{k_B T}.$$  \hspace{1cm} (S12)

Here $\mathbb{1}$ corresponds to the unity matrix, $p_H$ is Boltzmann population factor, $\gamma_H$ is proton gyromagnetic ratio, $k_B$ the Boltzmann constant, and $T$ stands for the (spin) temperature, which can be lower than the sample temperature if DNP or other hyperpolarization methods are used. By choosing a normalization factor of 1/16, the total magnetization of all 4 spins is 1. The yield of the excitation and reconversion of LLS during single and double SLIC experiments can be readily calculated. The amplitude $c_1$ of an operator $\hat{P}$ contained in a density operator $\hat{\sigma}$ is defined according to:

$$\hat{\sigma} = c_1 \hat{P} + \text{orthogonal operators.}$$  \hspace{1cm} (S13)

and can be calculated as:

$$c_1 = \frac{\langle \hat{P} | \hat{\sigma} \rangle}{\langle \hat{P} | \hat{P} \rangle} = \frac{\text{Tr}[\hat{\rho}^\dagger \hat{\sigma}]}{\text{Tr}[\hat{\rho}^\dagger \hat{\rho}]}.$$  \hspace{1cm} (S14)

The excitation by a 90° pulse followed by single or double-S LIC transfers either a population of $\pm p_H/16$ or $\pm p_H/8$ of the total magnetization from a corresponding triplet state (either $T_i^{A^A'T^{XX'}}$ for single SLIC applied to AA’, or $T_i^{A^A'T^{XX'}}$ for single SLIC applied to XX’, or $T_{i \pm 1}^{A^A'T^{XX'}}$ for double SLIC) into the $S_0^{A^A'S^{XX'}}$ state as illustrated in Fig. S2a and Fig. S2b. This creates long-lived population imbalances with amplitudes of $\pm \left( \frac{1}{9} + 1 \right) \frac{1}{16} p_H$ or $\pm \left( \frac{1}{9} + 1 \right) \frac{1}{8} p_H$ for single or double SLIC respectively.

We assume then that all short-lived components are removed by relaxation, the gradients of the $T_{00}$ filters, and phase cycling, so that all population imbalances between triplet states are eliminated as illustrated in Fig. S2c and Fig. S2d. The imbalances are assumed to be fully preserved, as expected for short relaxation times $\tau_{\text{rel}}$. Therefore, the average populations of the nine $T_i^{A^A'T^{XX'}}$ triplet states corresponds to $\pm \frac{1}{9} \frac{1}{16} p_H$ or $\pm \frac{1}{9} \frac{1}{8} p_H$ for single or double SLIC, respectively, whereas the population of the $S_0^{A^A'S^{XX'}}$ state is either $\pm \frac{1}{16} p_H$ or $\pm \frac{1}{8} p_H$ for single or double SLIC. Finally, the transformation of LLS into observable magnetization by the reconversion SLIC pulses swaps populations of one of the triplet-triplet states and the singlet-singlet state in the tilted frame, so that the final state in the laboratory frame corresponds to transverse magnetization with a phase that is parallel to the phase of SLIC pulses. These coherences are illustrated in Fig. S2e and Fig. S2f. The amplitudes of these coherences, calculated using Eq. (S14), are 5/72 of the total initial magnetization for single SLIC excitation and reconversion, 5/36 for double SLIC excitation and single SLIC reconversion, and 5/18 for double SLIC excitation and reconversion. According to unitary constraints (50) modified to take spin symmetry into account (51) it can be shown that 5/18
is the maximum achievable yield of conversion of magnetization into the LLS and back. Experimentally however, the observed yields are typically 3 times lower. We attribute this to the inhomogeneity of the RF field that has a rather dramatic effect on the yields especially for double SLIC (55).

Fig. S2. Energy level diagrams showing populations and coherences after single SLIC (left) or double SLIC (right). The open and filled symbols in (a, b) show excesses and deficiencies of populations at thermal equilibrium with respect to the demagnetized (saturated) state. If the RF phase of the single or double SLIC irradiation is applied along the +x axis after the initial of 90° pulse, this allows one to swap the populations of the $T_{AA'}^0 T_0^{XX'}$ and $S_0^{AA'} S_0^{XX'}$ states for single SLIC, or of the $T_{AA'}^1 T_{XX'}^1$ and $S_0^{AA'} S_0^{XX'}$ states for double SLIC (shown as double-headed solid arrows). If the RF phase is along the -x axis, one can swap the populations of $T_{AA'}^{-1} T_0^{XX'}$ or $T_{AA'}^{-1} T_{XX'}^{-1}$ with the populations of $S_0^{AA'} S_0^{XX'}$ (shown as double-headed dashed arrows), as can be described by “singlet order selection” (SOS, ref. (18)). (c, d) Distribution of populations in the laboratory frame after LLS excitation, assuming that the $S_0^{AA'} S_0^{XX'}$ state was overpopulated. The 9 triplet-triplet product states are therefore depleted, whereas the 3 singlet-triplet and 3 triplet-singlet states on the right-hand side are saturated, i.e., have neither excess nor deficient populations. Note that yield achieved by double SLIC is twice as large as for single SLIC. (e, f) Single-quantum coherences excited after reconversion of LLS by single or double SLIC. Single SLIC applied to the AA’ spins excites two degenerate coherences shown in purple. Double SLIC excites six transitions that lead to 1:2:1 triplets for both AA’ and XX’ spins.
Effects of strong second-order couplings on LLS excitation in AA'BB' systems

The theory described above is valid for weakly-coupled AA’XX’ systems where the difference between resonance frequencies of the AA’ and XX’ spins is much larger than the vicinal couplings $J_{AA'}$ and $J_{AX'}$. This assumption may not hold, especially at low fields, and one may have strongly coupled spin systems that can be denoted in Pople’s notation by AA’BB’ (46). In the absence of any RF irradiation, the unperturbed Hamiltonian written in a frame rotating at the mean chemical shift frequency of the AA’ and BB’ spin pairs is then given by:

$$
\hat{H} = J_{AA'} \hat{I}^A \cdot \hat{I}'^A + J_{XX'} \hat{I}^X \cdot \hat{I}'^X + \frac{1}{2} \sum (\hat{I}^A + \hat{I}'^A) \cdot (\hat{I}^X + \hat{I}'^X) + \frac{1}{2} \Delta J (\hat{I}^A - \hat{I}'^A) \cdot (\hat{I}^X - \hat{I}'^X) + \frac{\Delta \nu}{2} (\hat{I}^z_A + \hat{I}'^z_A - \hat{I}^z_X - \hat{I}'^z_X).
$$

(S15)

Numerical simulations of the excitation of LLS by single and double SLIC for AA’BB’ spin systems show the yield of LLS excitation as function of $\Delta \nu = \nu_A - \nu_X$ and of $\Delta J$ (Fig. S3).

Fig. S3. Strong-coupling effects on the LLS excitation yields in AA’BB’ systems. Yield of LLS excitation achieved by single SLIC (a) and double SLIC (b) excitation as a function of the difference of chemical shifts $\Delta \nu$, and the difference between the vicinal J-couplings $\Delta J$. The simulations were performed for $J_{AA'} = -13$ Hz, $J_{XX'} = -14$ Hz, $\Sigma J = 14$ Hz, with 100 steps of $\Delta \nu$ from 1 to 250 Hz, and 8 steps of $\Delta J$ from 1.5 Hz to 6 Hz.

One can see that for both single and double SLIC the plots are similar: all curves essentially lie on a flat plateau that corresponds to the maximum possible yield for $\Delta \nu > 100$ Hz. For systems with $\Delta J < 2$ Hz, the yield decreases more quickly upon decreasing of $\Delta \nu$ than for $\Delta J > 2$ Hz. The critical value of $\Delta \nu$ below which SLIC fails is about $\Delta \nu = 60$ Hz for $\Delta J > 2$ Hz and about $\Delta \nu = 80$ Hz for $\Delta J < 2$ Hz. The range of $\Delta J$ shown corresponds to typical experimental values in Table 1 of the main text.

Rotamer populations and vicinal J-couplings

The value of $\Delta J$, which is crucial for the excitation and reconversion of LLS, depends on the populations of the rotamers shown in Fig. S4. For alkanes in solution, the averaged value of vicinal $^2J_{AX}$ and $^2J_{AX'}$ couplings can be calculated in terms of the populations of the three rotamers and of the values of the trans $^2J(180^\circ)$-coupling and gauche $^2J(60^\circ)$- and $^2J(300^\circ)$-couplings that are equal in achiral molecules:

$$
\langle ^2J_{AX} \rangle = ^2J_{HH}(180^\circ) \cdot P_t + ^2J_{HH}(60^\circ) \cdot (P_{II} + P_{HH}),
$$

$$
\langle ^2J_{AX'} \rangle = ^2J_{HH}(60^\circ) \cdot (P_t + P_{HH}) + ^2J_{HH}(180^\circ) \cdot P_{II}.
$$

(S16)
The sum of all populations $P_I + P_{II} + P_{III} = 1$, with $P_{II} = P_{III}$ in achiral molecules. The difference $\Delta J$ is given by:

$$\Delta J = \langle 2J_{AX} \rangle - \langle 2J_{AX'} \rangle = \frac{3}{2}(2J_{HH}(60^\circ) - 2J_{HH}(180^\circ)) \cdot \delta,$$

(S17)

Where $\delta$ corresponds to the overpopulation of $P_I = \frac{1}{3} + \delta$ that can vary in the range $-1/3 \leq \delta \leq 2/3$.

Fig. S4. Newman projections of the three rotamers with lowest energies in a CH$_2$R$_1$-CH$_2$R$_2$ fragment. The four protons are labeled as AA'XX'. The green arrows connect spins A and X whereas the blue arrows connect spins A and X'. In rotamer I, which has a plane of symmetry in achiral molecules, the substituents R$_1$ and R$_2$ are in an antiperiplanar configuration, while rotamers II and III correspond to gauche configurations, which are equally populated in achiral molecules.

The values of vicinal couplings are constrained by Karplus’ relationships:

$$2J_{HH}(\varphi) = a \cos^2 \left(2\pi \frac{\varphi}{360}\right) + b \cos \left(2\pi \frac{\varphi}{360}\right) + c,$$

(S18)

Where the coefficients $a$, $b$, $c$ can be determined empirically. By substituting eq. (S18) into (S17) one obtains:

$$\Delta J = \frac{9}{8}(2b - a) \cdot \delta.$$

(S19)

For the molecules studied in this work we observed $\Delta J$ in range of $0 \leq \Delta J \leq 6.4$ Hz. $\Delta J = 0.8$ Hz is the smallest difference where we found LLS to still be accessible. From the literature (47, 48) one can estimate the difference of the Karplus coefficients to lie in the range between $11 \leq |2b - a| \leq 15$ Hz. Therefore, the smallest value of $|\delta|$ that allows the LLS to be accessible can be estimated to be $0.05 \leq |\delta| \leq 0.06$, as in the case of $\beta$-mercaptoethanol. For the largest $\Delta J = 6.4$ Hz that was observed in DSS, it appears that $0.4 \leq |\delta| \leq 0.5$, close to its maximum possible value $\delta = 0.66$.

Practical recommendations for setting up single- and double-SLIC experiments

To maximize the yield of the conversion from magnetization to LLS by single or poly-SLIC in aliphatic systems, the pulse amplitude $v_{RF}$ and duration $\tau_{SLIC}$ can be adjusted empirically, bearing in mind that the $J$-couplings are generally not known with sufficient accuracy. In AA’XX’ systems, the ideal amplitude of a single SLIC pulse corresponds to the sum of the two geminal $J$-couplings: $v_{RF} = |2J_{AA'} + 2J_{XX'}|$. 
In favourable cases, the optimum RF amplitude $v_{RF}$ can be deduced directly from the appearance of the multiplet. In a multiplet characteristic of an AA’XX’ system, the distance between the weak outer ‘combination lines’ (see Fig. S5) is approximately $2|J_{AA'} + J_{XX'}|$. If these outer lines are too weak to be visible in the spectrum, one can use a trial SLIC pulse to test whether the outer transitions can be enhanced (66). For a trial SLIC pulse, we can choose an amplitude $v_{RF} = 27$ Hz, corresponding to twice the value of a typical geminal coupling constant $J_{AA'}$ in aliphatic systems and use it as an excitation pulse applied at resonance frequency of the CH$_2$ signal.

The optimum duration of a single SLIC pulse $\tau_{SLIC}$ is inversely related to $\Delta J$ by the following equation:

$$\tau_{SLIC} = \frac{1}{\sqrt{2|\Delta J|}}.$$  

(S20)

---

**Fig. S5. Simulated multiplet of an AA’XX’ system.** The positions of the combination lines are indicated with asterisks.

**Fig. S6. Experimental multiplets of methylene protons with small and more pronounced magnetic inequivalence.** (a) Appearance of a multiplet in taurine. The optimized $\tau_{SLIC}$ is 520 ms in this case, corresponding to a small $\Delta J$ value of 1.4 Hz. (b) Appearance of a multiplet in DSS. The optimized $\tau_{SLIC}$ is 110 ms, corresponding to a larger $\Delta J$ of 6.4 Hz.

In principle, the value of $\Delta J$ can also be derived from the appearance of the multiplet; in practice however, the spectrum may not be sufficiently well resolved. Table 1 of the main text shows $\tau_{SLIC}$ values ranging from 110 to 845 ms, reflecting $\Delta J$ values of 6.4 to 0.8 Hz. If the multiplet resembles a first-order triplet (as in taurine, see Fig. S6a) $\Delta J$ is likely to be small and the initial trial duration should be rather long, say $\tau_{SLIC} = 500$ ms. If we observe a typical second-order AA’XX’ pattern (as in DSS, see Fig. S6b), $\Delta J$ is likely to be larger, and a reasonable initial trial value would be $\tau_{SLIC} = 100$ ms.
Once the parameters of the trial SLIC pulse have been chosen, the RF carrier should be set to the center of the multiplet and a simple 1D (zg) experiment recorded using the trial SLIC pulse as an excitation pulse. Compare the resulting spectrum with a conventional spectrum (excited with a non-selective 90° pulse) recorded under the same conditions. If the trial parameters of the SLIC pulse are appropriately chosen, one should see an enhancement of the outer combination lines compared to those in the conventional spectrum (see Fig. S7). The distance of these lines from the center of the multiplet can then be used to estimate $v_{RF}$ of SLIC pulse. For systems with very small $\Delta J$ a large number of scans may be necessary to see enhancement of the combination lines.

![Fig. S7. Enhancement of the combination lines by application of a trial SLIC pulse.](image)

Now that the optimum RF amplitude has been determined, one can use the parameter optimization tool “popt” of Bruker’s TopSpin software to optimize the duration $\tau_{SLIC}$. For this, one should use a one-dimensional SLIC experiment with a fixed delay $\tau_{rel}$. Ensure that the digital resolution is sufficient and optimize the integral of the entire multiplet rather than then the highest peak. Fig. S8 shows a typical result of such an optimization. Additional iterations of “popt” can be used to optimize $v_{RF}$ and $\tau_{SLIC}$ further.

![Fig. S8. Optimization of the pulse duration $\tau_{SLIC}$ for GABA.](image)

If one wishes to use poly-SLIC on neighboring CH$_2$ groups, the parameters $v_{RF}$ and $\tau_{SLIC}$ must be adapted as follows: $\tau_{polySLIC} = \sqrt{2} \tau_{SLIC}$, $v_{RF} polySLIC = \frac{v_{RF}}{2}$. 
For poly-SLIC, one must selectively irradiate several offsets at the same time. To generate a pulse that does this, we use the TopSpin ShapeTool (see Fig. S9) to create shaped SLIC pulses.

I) Type `stdisp` in the TopSpin command line to open the ShapeTool display.

II) From the menu of shapes, choose the waveform “Rectangle”. Set the number of points so that shape is smooth over the entire pulse length $\tau_{SLIC}$.

III) Click on the pen symbol on the left and choose “multiple phase modulation”.
   
   a. When creating a SLIC pulse to convert magnetization to LLS, choose “Alignment: beginning at phase 0”. When creating a SLIC pulse to reconvert LLS into magnetization, choose “Alignment: ending at phase 0”.
   
   b. The option “reference frequency: no reference frequency specified” will take the later specified offsets relative to the offset O1 set in the acquisition parameters.
   
   c. Enter the frequencies that must be irradiated in Hz. In the example shown in Figure S9, the first three items in the frequency list are set to 0, -565.25 and 574.98 Hz, which is appropriate for triple SLIC applied to DSS at a field of 11.5 T (500 MHz). This means that three RF frequencies will be applied simultaneously at O1, at 565.25 below and 574.98 above O1. The O1 will be set to the frequency of the middle multiplet of DSS in the acquisition parameters.
   
   d. Save the created shaped pulse and copy it into the acquisition parameters. Set the RF amplitude and duration of the shaped pulse. Note that RF power should be scaled according to the number of irradiated multiplets. In the example shown in Figure S9, triple irradiation is used and therefore the power for the shaped pulse should be set to $3 \times 14 = 42$ Hz in order to have a $\nu_{RF}$ of 14 Hz at each offset.
Poly-SLIC pulse sequence

; pulse sequence for singlet order decay measurement using (poly-)SLIC
; AVANCE NEO and AVANCE III
; kirill.sheberstov@ens.psl.eu

#include <Avance.incl>
#include <Grad.incl>
define list<delay> vd_list=$VDLIST

"d12=20u"
1 ze
2 d1
   10u pl1:f1
   p1 ph1
   10u pl0:f1
   (p3:sp1 ph2):f1

   vd_list
   50u UNBLKGRAD
   p16:gp1
d16
   10u pl1:f1
   p1 ph=90.0
d12
   p17:gp2
d16
   p1 ph=54.7
d12
   p1 ph=0.0
d12
   p18:gp3
d16
   4u BLKGRAD

   10u pl0:f1
   (p4:sp2 ph3):f1

   go=2 ph31
   30m wr #0 if #0 vd_list.inc
lo to 1 times td1
exit

ph1=0 0 0 0
ph2=1 3 1 3
ph3=1 1 3 3
ph31=0 2 2 0

;pl1 : f1 channel - power level for pulse (default)
;p1 : f1 channel - 90 degree high power pulse
;p3 : duration of first shaped SLIC pulse (excitation)
;p4 : duration of second shaped SLIC pulse (reconversion)
;pl0 : set to 0 Watt
;sp1 : shaped SLIC pulse used for excitation of LLS
;sp2 : shaped SLIC pulse used for reconversion of LLS
;p16: first gradient in T00 filter
;p17: second gradient in T00 filter
;p18: third gradient in T00 filter
;vd : list of delays to sustain singlet order
;d12: delay for power switching                      [20 usec]
;d16: delay for homospoil/gradient recovery