Low-Frequency Excitation of Singlet-Triplet Transitions.
Application to Nuclear Hyperpolarization

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Coupled pairs of nuclear spins-1/2 support one singlet state and three triplet states. Transitions between the singlet state and one of the triplet states may be driven by an oscillating low-frequency magnetic field, in the presence of couplings to a third nuclear spin, and a weak bias magnetic field. This phenomenon allows the generation of strong nuclear hyperpolarization of $^{13}$C nuclei, starting from the nuclear singlet polarization of a $^3$H spin pair, associated with the enriched para spin isomer of hydrogen gas. Hyperpolarization is demonstrated for two molecular systems.

I. INTRODUCTION

Magnetic resonance experiments usually involve the application of a strong magnetic field (typically, many Tesla) combined with radiofrequency pulses (for nuclear magnetic resonance, NMR) or microwave pulses (for electron spin resonance, ESR) that are resonant with the magnetic Zeeman transitions of the system. In these high-field conditions, the parts of the spin Hamiltonian that do not commute with the Zeeman Hamiltonian are usually removed. This secular approximation leads to a major simplification of spin dynamical theory and is one of the cornerstones of modern NMR theory [1]. One consequence is that all transitions involving combinations or multiples of the Larmor frequencies are not observed in NMR or ESR spectra. Some exceptions to this paradigm exist, such as “overtone” transitions in the NMR of nuclei with large quadrupolar moments [2], and the use of non-secular hyperfine couplings in solid-effect dynamic nuclear polarization [3].

In the case of NMR, the secular approximation breaks down in very small magnetic fields, such that the Larmor frequencies are comparable in magnitude to the spin-spin interactions [4]. Here we show that the mixing of states by non-secular spin-spin couplings in the low-field regime allows selected “forbidden” transitions to be induced by oscillating magnetic fields. In suitable circumstances, this phenomenon allows the generation of strong hyperpolarization in the reaction products of hydrogen gas enriched in the para spin isomer. We demonstrate the generation of two molecules in solution with high levels of $^{13}$C polarization. One of these substances, fumarate, is a natural metabolite which has been used for the characterisation of cancer in magnetic resonance imaging (MRI) [5].

II. THEORY

Consider an ensemble of nuclear three-spin-1/2 systems, each consisting of two nuclei $I_1$ and $I_2$ of one isotopic type with magnetogyric ratio $\gamma_I$, and a third nucleus $S_3$ of a different isotopic type with magnetogyric ratio $\gamma_S$. In the isotropic solution state, the three nuclei mutually interact by scalar spin-spin coupling terms mediated by the bonding electrons. These couplings consist of a homonuclear coupling $J_{12}$, and two heteronuclear couplings $J_{13}$ and $J_{23}$. We assume that the two heteronuclear couplings are unequal, $J_{13} \neq J_{23}$.

A. WOLF pulses

A small magnetic field $B_z$ is applied along the laboratory frame $z$-axis. This field is the sum of a time-independent “bias” field $B_{\text{bias}}$, and a time-dependent oscillating field denoted $B_{\text{WOLF}}$, such that $B_z(t) = B_{\text{bias}} + B_{\text{WOLF}}(t)$. The oscillating field is given by

$$B_{\text{WOLF}}(t) = B_{\text{WOLF}}^0 \cos(\omega_{\text{WOLF}} t),$$

where $\omega_{\text{WOLF}}$ is the frequency of the oscillating field, and $B_{\text{WOLF}}^0$ is its peak amplitude. Since the applied oscillating field is small in magnitude and low in frequency, we refer to the oscillating magnetic field as a WOLF (Weak Oscillating Low Frequency) pulse.

![Approximate energy level diagram of a system of two I-spins and one S-spin, in the near-equivalence limit $(|J_{13} - J_{23}| \ll |J_{12}|)$. The circles represent the population distribution for a fully populated singlet state between the two I-spins. The Larmor frequencies in the bias field $B_{\text{bias}}$ are indicated: $\omega_I^0 = -\gamma_I B_{\text{bias}}$ for the I-spins, and $\omega_S^0 = -\gamma_S B_{\text{bias}}$ for the S-spins. The WOLF pulse is applied with a frequency $\omega_{\text{WOLF}}$ matching the transition frequency $\omega_{ST}$ between the indicated pair of states (equation 7).](attachment:figure1.png)
Note that the $B_{\text{bias}}$ and $B_{\text{WOLF}}$ fields are applied in the same direction. This geometry differs from most NMR experiments, where oscillating fields are applied transverse to the main field, with few exceptions [2, 6, 7].

If the magnetic fields are low enough that chemical shifts are negligible, the spin Hamiltonian may be written as a sum of five terms

$$H(t) = H_A + H_B + H_C + H_D + H_{\text{WOLF}}(t).$$

These are given by

$$H_A = -B_{\text{bias}} \left( \gamma_I (I_{1z} + I_{2z}) + \gamma_S S_{3z} \right) + 2\pi J_{12} I_1 \cdot I_2 + \pi (J_{13} + J_{23})(I_{1z} + I_{2z}) S_{3z},$$

$$H_B = \pi (J_{13} - J_{23})(I_{1z} - I_{2z}) S_{3z},$$

$$H_C = \frac{1}{2} \pi (J_{13} + J_{23}) \times \left( I_1^3 S_3^- + I_2^3 S_3^+ + I_1^3 S_3^+ + I_2^3 S_3^- \right),$$

$$H_D = \frac{1}{2} \pi (J_{13} - J_{23}) \times \left( I_1^3 S_3^- - I_2^3 S_3^+ + I_1^3 S_3^+ - I_2^3 S_3^- \right),$$

$$H_{\text{WOLF}}(t) = -B_{\text{WOLF}}(t) \left( \gamma_I (I_{1z} + I_{2z}) + \gamma_S S_{3z} \right).$$

The terms $H_A$, $H_C$, and $H_{\text{WOLF}}$ are symmetric with respect to exchange of the two $I$-spins, while the terms $H_B$ and $H_D$ are antisymmetric. If the Larmor frequency of the nuclei in the bias field is larger than the spin-spin couplings, and the difference between the heteronuclear couplings is smaller than the homonuclear coupling ($|J_{13} - J_{23}| \ll |J_{12}|$, i.e. near-equivalence [8]), then the $H_A$ term dominates. The terms $H_C$ and $H_{\text{WOLF}}$ commute with $H_A$ and may be regarded as secular, while the terms $H_B$ and $H_D$ do not commute with $H_A$ and are non-secular. The terms $H_B$ and $H_C$ both give rise to small eigenvalue shifts, which are neglected here, for the sake of brevity. The exchange-antisymmetric non-secular term $H_D$ is the most important one for the purposes of this paper.

The eigenstates and eigenvalues of $H_A$ are sketched in figure 1. The eigenstates are direct products of the singlet and triplet states of the $I$-spin pair with the Zeeman states of the $S$-spin, such that the triplet product states are symmetric and the singlet product states are antisymmetric under permutation of spins 1 and 2:

$$|T_M m_S \rangle = (+1)|T_0 m_S \rangle,$$

$$|S_0 m_S \rangle = (-1)|S_0 m_S \rangle,$$

where $M \in \{-1, 0, 1\}$ and the Zeeman eigenequations are

$$|I_{1z} + I_{2z}|T_M m_S \rangle = M|T_M m_S \rangle,$$

$$|I_{1z} + I_{2z}|S_0 m_S \rangle = 0,$$

$$S_{4z}|T_M m_S \rangle = m_S|T_M m_S \rangle,$$

$$S_{3z}|S_0 m_S \rangle = m_S|S_0 m_S \rangle.$$  

The symbols $\alpha$ and $\beta$ are used to denote $m_S = \pm \frac{1}{2}$, respectively.

Consider the “forbidden” transition between the $|S_0 \beta \rangle$ and $|T_{-1} \alpha \rangle$ state, indicated in figure 1. All Hamiltonian terms in equation 3 have a zero matrix element connecting these two states, except for the non-secular antisymmetric term $H_D$, with matrix elements given by

$$\langle S_0 \beta | H_D | T_{-1} \alpha \rangle = \langle T_{-1} \alpha | H_D | S_0 \beta \rangle = 2^{-1/2} \pi (J_{13} - J_{23}).$$

The difference between the corresponding diagonal elements of $H_A$ is given by

$$\omega_{ST} = \langle T_{-1} \alpha | H_0 | T_{-1} \alpha \rangle - \langle S_0 \beta | H_0 | S_0 \beta \rangle = B_{\text{bias}}(\gamma_I - \gamma_S) + \frac{\pi}{2}(4J_{12} - J_{13} - J_{23}),$$

where the notation $\omega_{ST}$ indicates “singlet-triplet transition”. Note that this transition frequency includes a combination of $I$-spin and $S$-spin Larmor frequencies.

The term $H_D$ induces a slight mixing of these two $H_A$ eigenstates. The small degree of state mixing would have very little effect, if it were not for the time-dependence introduced by the coupling to the oscillating magnetic field, $H_{\text{WOLF}}(t)$. If the oscillation frequency matches the singlet-triplet transition frequency ($\omega_{\text{WOLF}} \approx \omega_{ST}$), the periodic time-dependence drives coherent transitions between these two eigenstates. For example, suppose the initial state of the system consists of a strongly populated state $|S_0 \beta \rangle$, and a completely depleted state $|T_{-1} \alpha \rangle$. The density operator is described by

$$\langle S_0 \beta | \rho(0) | S_0 \beta \rangle = 1,$$

$$\langle T_{-1} \alpha | \rho(0) | T_{-1} \alpha \rangle = 0.$$  

Consider a WOLF pulse applied for a duration $\tau$, on the resonance condition $\omega_{\text{WOLF}} = \omega_{ST}$. As shown in the Supplemental Material [9], the spin-state populations after the pulse are approximately given by

$$\langle S_0 \beta | \rho(\tau) | S_0 \beta \rangle \simeq \frac{1}{2} (1 + \cos(\omega_{\text{mut}}^{ST} \tau)),$$

$$\langle T_{-1} \alpha | \rho(\tau) | T_{-1} \alpha \rangle \simeq \frac{1}{2} (1 - \cos(\omega_{\text{mut}}^{ST} \tau)),$$

where the singlet-triplet mutation frequency under the WOLF pulse is given by

$$\omega_{\text{mut}}^{ST} = 2\pi \sqrt{J_{12}^2 + J_{23}^2} \sin(\phi + \theta/2)J_1(A).$$

The angles $\theta$ and $\phi$ are given by

$$\theta = \arctan(2J_{12}, J_{13} - J_{23}),$$

$$\phi = \arctan(2J_{13} + J_{23}, J_{13} - J_{23}),$$

such that $\theta, \phi \ll 1$ in the near-equivalence regime.

The symbol $J_1$ denotes a Bessel function of the first kind with its argument given by

$$A = (\gamma_I - \gamma_S)B_{\text{WOLF}}^0/\omega_{ST}.$$  

These equations apply approximately in the limits $|J_{13} - J_{23}| \ll |J_{12}|$ and $J_{13}^2 + J_{23}^2 \ll \omega_{ST}^2$.

It is therefore possible to transport population completely from one state to the other by applying a WOLF
pulse of duration $\pi/\omega_{\text{out}}^{\text{ST}}$. The inversion speed is maximised by choosing the peak amplitude of the WOLF pulse to equal $B_0^0 \approx 2B_{\text{bias}}$, at which point the Bessel function $J_1(A)$ reaches its approximate maximum. Since there are practical limits on the generation of large oscillating magnetic fields, this technique is most appropriate for low-field magnetic resonance.

The use of oscillating magnetic fields for the selective excitation of transitions has previously been explored in the context of ultralow-field NMR [7]. However, those experiments follow the familiar paradigm of resonant excitation, in which transitions are induced by modulating off-diagonal Hamiltonian terms at a frequency that matches the transition energy. In the current case, on the other hand, the off-diagonal non-secular terms are time-independent, and it is the diagonal terms that are given a periodic time-dependence by the oscillating applied field. There is a distant relationship with selective excitation in magic-angle-spinning solid-state NMR [10].

### B. Parahydrogen-induced polarization

The resonant driving of singlet-triplet transitions is particularly useful in the context of parahydrogen-induced polarization (PHIP), a technique which is widely used to enhance NMR signals [11–25]. In this method, hydrogen gas is enriched in the para spin isomer and reacted with a substrate in the presence of a catalyst. The proton pair of the product molecule exhibits excess population in the singlet state. The strongly enhanced singlet spin order is converted into hyperpolarized magnetization of heteronuclei such as $^{13}$C by applying a sequence of magnetic fields. A range of suitable techniques has been developed [13, 15–18, 20–22, 26, 27].

We now show that the application of a WOLF pulse in a small bias magnetic field leads to strong hyperpolarization of $^{13}$C nuclei in the reaction products of para-enriched H$_2$ gas, with potential advantages over other methods, as discussed below.

The principle of the experiment is shown in figure 1. The spin state populations (indicated by balls) are given for the case that the spins $I_1$ and $I_2$ are protons originating from the para-enriched hydrogen and the spin $S_3$ is a $^{13}$C nucleus in the product molecule. The populations of the $|S_0\alpha\rangle$ and $|S_0\beta\rangle$ states are strongly enhanced due to their provenance as the nuclei of the para-enriched H$_2$ spin isomer. Since these two populations are equal there is no polarization of the $S$-spin at this stage. However, if the WOLF pulse transports the population from the $|S_0\beta\rangle$ state to the $|T-\alpha\rangle$ state, as shown in equation 9, the resulting population distribution has excess population in the $|S_0\alpha\rangle$ and $|T-\alpha\rangle$ states. Since the $S$-spin is in the state $|\alpha\rangle$ in both cases, this corresponds to a high degree of $^{13}$C polarization. A $^{13}$C polarization of the order of unity represents an enhancement of the $^{13}$C NMR signals by around 5 orders of magnitude, relative to ordinary NMR based on thermal equilibrium polarization in a strong magnetic field.

We propose the acronym WEREWOLF (Whopping Enhancement Requires Excitation by Weak Oscillating Low Fields) for para-hydrogen-induced polarization of heteronuclei using WOLF pulses for the singlet-triplet population transfer.

![Figure 2](https://via.placeholder.com/150)

**FIG. 2.** $^1$H-decoupled $^{13}$C spectra of (a) fumarate and (b) maleate at a field of 9.41 T. Single-transient WEREWOLF-hyperpolarized $^{13}$C spectra are compared with conventional $^{13}$C NMR spectra acquired at thermal equilibrium, averaged over 360 transients (for fumarate) or 512 transients (for maleate). The strong $^{13}$C peaks are from hyperpolarized naturally occurring $^{13}$C nuclei at the molecular sites indicated by filled circles. The $^{13}$C peaks at 160 ppm and 76 ppm in (a) correspond to unreacted disodium acetylene dicarboxylate. The small signal at 172 ppm in (b) is attributed to succinate generated by secondary hydrogenation.

| Compound     | $J_{12}(\text{Hz})$ | $J_{23}(\text{Hz})$ | $J_{13}(\text{Hz})$ |
|--------------|---------------------|---------------------|---------------------|
| Fumarate     | 15.9                | 5.8                 | 3.3                 |
| Maleate      | 12.3                | 12.9                | 2.5                 |

### III. METHODS

#### A. Materials

The substances fumarate (E-butenedioate) and maleate (Z-butenedioate) were used for demonstrations of WEREWOLF. The chemical structures are shown in figure 2. Both compounds are formed by the catalytic hydrogenation of acetylene dicarboxylate, using a ruthenium-based catalyst for fumarate [19], and a
rhodium-based catalyst for maleate [21]. Chemical details are given in the Supplemental Material [9].

About 2% of fumarate and maleate molecules contain a naturally-occurring $^{13}$C nucleus at the sites shown in figure 2. The two $^1$H nuclei and the $^{13}$C nucleus form a three-spin-1/2 system of the type discussed above. The $J$-coupling parameters for the two molecular systems are given in table I.

Para-enriched hydrogen was produced by passing hydrogen gas over an iron oxide catalyst cooled by liquid nitrogen.

### B. Equipment

A sketch of the equipment is shown in figure 3. This consists of a mu-metal chamber for magnetic shielding, equipped with a solenoid coil (for the WOLF pulses) and a Helmholtz coil (for the bias field). A guide coil allows the sample to traverse the chamber walls without passing through zero magnetic field. The coils are driven by waveform generator and stable current sources. The equipment set also includes computer-actuated valves and an NMR tube equipped with a capillary, allowing bubbling of para-enriched H$_2$ gas under microprocessor control. Full details are in the Supplemental Material [9].

### C. Experimental Procedure

Figure 4 shows a timing diagram of the WEREWOLF experiment, showing the magnetic fields experienced by the sample as a function of time. Each experiment starts by warming the sample mixture to $\sim 90^\circ$C in the ambient magnetic field of the laboratory ($\sim 110 \mu$T), followed by insertion into the magnetic shield. Para-enriched hydrogen gas is bubbled through the solution at 6 bar pressure. The bias field is reduced to $B_{bias} = 2 \mu$T while
the oscillating WOLF pulse is applied with amplitude \( B_0^{WOLF} = 2 \mu T \) for an interval \( \tau \). The sample is removed from the shield and inserted by hand into the 9.41 T NMR magnet where a 90° \(^{13}\text{C} \) radio-frequency is applied. The \(^{13}\text{C} \) free-induction decay is acquired in the presence of \(^1\text{H} \) decoupling. Fourier transformation of the NMR signal gives the \(^{13}\text{C} \) NMR spectrum. Full details are in the Supplemental Material [9].

Comparison of these spectra allows the estimation of the \(^{13}\text{C} \) polarization levels achieved by WEREWOLF, which are \( p \approx 8\% \) for fumarate and \( p \approx 19\% \) for maleate. These results are highly competitive with previous work [24, 29, 30], especially when the relatively low \( \text{para} \)-hydrogen enrichment levels, crude apparatus, manual sample transport, and sub-optimal reaction conditions are taken into account.

Integrated \(^{13}\text{C} \) signal amplitudes as a function of the WOLF pulse duration \( \tau \) are shown in figure 5. The coherent oscillations of the hyperpolarized magnetization are striking. Each experimental point was obtained from a separate experiment on a fresh sample. The normalized experimental data is compared with numerical simulations and analytical curves derived from equations 9 and 10. The agreement between both curves and the experimental data is gratifying.

### V. CONCLUSIONS

Magnetic resonance phenomena involving non-secular spin-spin couplings are normally encountered in systems with large nuclear quadrupolar couplings [2] or hyperfine couplings to unpaired electrons, as in solid-effect dynamic nuclear polarization (DNP) [3]. The work described here shows that non-secular couplings may also be exploited in purely nuclear spin systems, albeit at an energy scale which is \( \sim 9 \) orders of magnitude lower than in solid-effect DNP. Here too, non-secular effects allow “forbidden” transitions to couple to the electromagnetic field, allowing the generation of strong nuclear hyperpolarization.

There are several other techniques for performing the transformation of nuclear singlet order to heteronuclear magnetization, including pulse techniques in high field [13, 15, 17, 20, 27], field-cycling and level-anticrossing phenomena [16, 18, 21, 26], and low-field methods [18, 21, 25]. The WOLF method described here is highly intuitive, provides a very fast exchange of singlet and triplet populations, and has further potential advantages over current methods. Since the method works in the presence of a bias magnetic field, problems associated with ultralow-field NMR are avoided, such as short coherence lifetimes in the presence of rapidly relaxing nuclear species such as \(^2\text{H} \) and \(^{14}\text{N} \) [23]. The bias field resolves the Larmor frequencies of the different nuclear isotopes, making it possible to implement heteronuclear spin decoupling by resonant transverse oscillating fields. Furthermore, the coherent oscillatory spin evolution under the WOLF pulses suggests that the full palette of “pulse tricks” developed for conventional high-field NMR may be deployed in this regime, including error-compensating composite pulses [31] and adiabatic frequency sweeps [32]. Applications are envisaged to other hyperpolarization techniques, such as the SABRE (Signal Amplification by Reversible Exchange) method [14, 22]. The method described in the current paper might be re-
lated to a recently described variation of SABRE [25].

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[1] R. R. Ernst, G. Bodenhausen, and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions* (Clarendon Press, Oxford, 1987).
[2] R. Tycko and S. J. Opella, Overtone NMR spectroscopy, The Journal of Chemical Physics 86, 1761 (1987).
[3] W. T. Wenckebach, The Solid Effect, Applied Magnetic Resonance 34, 227 (2008).
[4] J. W. Blanchard, T. F. Sjolander, J. P. King, M. P. Ledbetter, E. H. Levine, V. S. Bajaj, D. Budker, and A. Pines, Measurement of untruncated nuclear spin interactions via zero- to ultralow-field nuclear magnetic resonance, Physical Review B 92, 220202 (2015).
[5] R. L. Hesketh and K. M. Brindle, Magnetic resonance imaging of cancer metabolism with hyperpolarized 13C-labeled cell metabolites, Current Opinion in Chemical Biology Molecular Imaging / Chemical Genetics and Epigenetics, 45, 187 (2018).
[6] G. Pileio, M. Caravetta, and M. H. Levitt, Extremely Low-Frequency Spectroscopy in Low-Field Nuclear Magnetic Resonance, Physical Review Letters 103, 083002 (2009).
[7] T. F. Sjolander, M. C. D. Tayler, J. P. King, D. Budker, and A. Pines, Transition-Selective Pulses in Zero-Field Nuclear Magnetic Resonance, The Journal of Physical Chemistry A 120, 4343 (2016).
[8] M. C. D. Tayler and M. H. Levitt, Singlet nuclear magnetic resonance of nearly-equivalent spins, Physical Chemistry Chemical Physics 13, 5556 (2011).
[9] See the Supplemental Material at [URL will be inserted by publisher] for experimental details and theoretical derivations.
[10] P. Caravatti, G. Bodenhausen, and R. R. Ernst, Selective experiments in high-resolution solid state NMR, Journal of Magnetic Resonance 55, 88 (1983).
[11] C. R. Bowers and D. P. Weitekamp, Transformation of Symmetrization Order to Nuclear-Spin Magnetization by Chemical Reaction and Nuclear Magnetic Resonance, Physical Review Letters 57, 2645 (1986).
[12] C. R. Bowers and D. P. Weitekamp, Parahydrogen and synthesis allow dramatically enhanced nuclear alignment, Journal of the American Chemical Society 109, 5541 (1987).
[13] M. Goldman and H. Jóhannesson, Conversion of a proton pair para order into 13C polarization by rf irradiation, for use in MRI, Comptes Rendus Physique 6, 575 (2005).
[14] R. W. Adams, J. A. Aguilar, K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, I. G. Khazal, J. Lopez-Serrano, and D. C. Williamson, Reversible Interactions with para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer, Science 323, 1708 (2009).
[15] S. Kadlecek, K. Emami, M. Ishii, and R. Rizi, Optimal transfer of spin-order between a singlet nuclear pair and a heteronucleus, Journal of Magnetic Resonance 205, 9 (2010).
[16] A. N. Pravdivtsev, A. V. Yurkovskaya, N. N. Lukzen, K. L. Ivanov, and H.-M. Vieth, Highly Efficient Polarization of Spin-1/2 Insensitive NMR Nuclei by Adiabatic Passage through Level Anticrossings, The Journal of Physical Chemistry Letters 5, 3421 (2014).
[17] T. Theis, M. Truong, A. M. Coffey, E. Y. Chekmenev, and W. S. Warren, LIGHT-SABRE enables efficient magnet catalytic hyperpolarization, Journal of Magnetic Resonance 248, 23 (2014).
[18] T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchebin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, and E. Y. Chekmenev, Microtesla SABRE Enables 10% Nitrogen-15 Nuclear Spin Polarization, Journal of the American Chemical Society 137, 1404 (2015).
[19] B. Ripka, J. Eills, H. Kourilová, M. Leutzsch, M. H. Levitt, and K. Münnemann, Hyperpolarized fumarate via parahydrogen, Chemical Communications 54, 12246 (2018).
[20] J. Eills, G. Stevanato, C. Bengs, S. Göggl, S. J. Elliott, J. Alonso-Valdesseiro, G. Pileio, and M. H. Levitt, Singlet order conversion and parahydrogen-induced hyperpolarization of 13C nuclei in near-equivalent spin systems, Journal of Magnetic Resonance 274, 163 (2017).
[21] J. Eills, J. W. Blanchard, T. Wu, C. Bengs, J. Hollenbach, D. Budker, and M. H. Levitt, Polarization transfer via field sweeping in parahydrogen-enhanced nuclear magnetic resonance, The Journal of Chemical Physics 150, 174202 (2019).
[22] D. A. Barskiy, S. Knecht, A. V. Yurkovskaya, and K. L. Ivanov, SABRE: Chemical kinetics and spin dynamics of the formation of hyperpolarization, Progress in Nuclear Magnetic Resonance Spectroscopy 114-115, 33 (2019).
[23] J. R. Birchall, M. S. H. Kabir, O. G. Salnikov, N. V. Chukanov, A. Svyatova, K. V. Kevtunov, I. V. Kopytyug, J. G. Gelovani, B. M. Goodson, W. Pham, and E. Y. Chekmenev, Quantifying the effects of quadrupolar sinks via15N relaxation dynamics in metronidazoles hyperpolarized via SABRE-SHEATH, Chemical Communications 56, 9098 (2020).
[24] S. Knecht, J. W. Blanchard, D. Barskiy, E. Cavallari, L. Dagys, E. V. Dyke, M. Tsukanov, B. Bliemel, K. Münnemann, S. Aime, F. Reineri, M. H. Levitt, G. Buntkowsky, A. Pines, P. Blümner, D. Budker, and J. Eills, Rapid hyperpolarization and purification of the metabolite fumarate in aqueous solution, Proceedings of the National Academy of Sciences 118 (2021).
[25] S. L. Ericksson, J. R. Lindale, X. Li, and W. S. Warren, Improving SABRE hyperpolarization with highly non-intuitive pulse sequences: Moving beyond avoided cross-
ings to describe dynamics, arXiv:2107.04687 [physics] (2021), arXiv:2107.04687 [physics].

[26] H. Jóhannesson, O. Axelsson, and M. Karlsson, Transfer of para-hydrogen spin order into polarization by diabatic field cycling, Comptes Rendus Physique Highly Polarized Nuclear Spin Systems and Dipolar Interactions in NMR, 5, 315 (2004).

[27] C. Bengs, L. Dagys, and M. H. Levitt, Robust transformation of singlet order into heteronuclear magnetisation over an extended coupling range, Journal of Magnetic Resonance 321, 106850 (2020).

[28] C. Bengs and M. H. Levitt, SpinDynamica: Symbolic and numerical magnetic resonance in a Mathematica environment, Magnetic Resonance in Chemistry 56, 374 (2018).

[29] B. A. Rodin, J. Eills, R. Picazo-Frutos, K. F. Sheberstov, D. Budker, and K. L. Ivanov, Constant-adiabaticity ultralow magneticfield manipulations of parahydrogen-inducedpolarization: Application to an AA’X spin system, Physical Chemistry Chemical Physics 23, 7125 (2021).

[30] B. A. Rodin, V. P. Kozinenko, A. S. Kryutin, A. V. Yurkovskaya, J. Eills, and K. L. Ivanov, Constant-adiabaticity pulse schemes for manipulating singlet order in 3-spin systems with weak magnetic non-equivalence, Journal of Magnetic Resonance 327, 106978 (2021).

[31] M. H. Levitt, Composite pulses, Progress in Nuclear Magnetic Resonance Spectroscopy 18, 61 (1986).

[32] J. Baum, R. Tycko, and A. Pines, Broadband and adiabatic inversion of a two-level system by phase-modulated pulses, Physical Review A 32, 3435 (1985).
Supplemental Material for Low-Frequency Excitation of Singlet-Triplet Transitions. Application to Nuclear Hyperpolarization

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I. WOLF PULSE DYNAMICS

The complete Hamiltonian given by equation 2 in the letter preserves the total $z$-angular momentum of the system

$$[H(t), I_z + I_{2z} + S_{3z}] = 0.$$  (1)

This suggests that the basis states highlighted in figure 1 separate the matrix representation of $H(t)$ into two $3 \times 3$ and two $1 \times 1$ blocks. The $1 \times 1$ blocks may be disregarded, the $3 \times 3$ blocks are generated by the sets

$$V = \{|S_0\alpha\}, |T_0\alpha\}, |T_+\beta\},$$

$$W = \{|S_0\beta\}, |T_0\beta\}, |T_-\alpha\}.$$  (2)

The standard WEREWOLF experiment (Fig. S1) aims to create positive heteronuclear magnetization which may be achieved through a population swap of states $|S_0\beta\rangle$ and $|T_-\alpha\rangle$. We thus consider the restriction of $H(t)$ to $W$, which may be explicitly given as follows

$$[H(t)]_W = \begin{bmatrix}
\frac{1}{2}(B(t)\gamma_S - 3\pi J_{12}) & \frac{\pi}{2}(J_{23} - J_{13}) & \frac{\pi}{2}(J_{13} - J_{23}) \\
\frac{\pi}{2}(J_{23} - J_{13}) & \frac{1}{2}(B(t)\gamma_S + \pi J_{12}) & \frac{\pi}{2}\sqrt{2}(J_{13} + J_{23}) \\
\frac{\pi}{\sqrt{2}}(J_{13} - J_{23}) & \frac{\pi}{\sqrt{2}}(J_{13} + J_{23}) & (\gamma_I - \frac{1}{2}\gamma_S)B(t) + \frac{\pi}{2}(J_{12} - J_{13} - J_{23})
\end{bmatrix},$$  (3)

where

$$B(t) = B_{\text{bias}} + B_{\text{WOLF}}\cos(\omega_{\text{WOLF}}t).$$  (4)

Although small, the mixing between the states $|S_0\alpha\rangle$ and $|T_0\alpha\rangle$ is not negligible during the WOLF pulse dynamics. To account for the mixing we define a set of rotated basis states

$$W_\theta = \{|S_0'\beta\}, |T_0\beta\}, |T_-\alpha\rangle\} = \{\cos(\theta/2)|S_0\beta\rangle + \sin(\theta/2)|T_0\beta\rangle, \cos(\theta/2)|T_0\beta\rangle - \sin(\theta/2)|S_0\beta\rangle, |T_-\alpha\rangle\},$$  (5)

parametrised by the angle $\theta$. The angle $\theta$ is chosen to satisfy

$$\theta = \arctan(2J_{12}, J_{13} - J_{23}).$$  (6)

In the near-equivalence regime the matrix representation of $H(t)$ restricted to $W_\theta$ is given by

$$[H(t)]_{W_\theta} \simeq \begin{bmatrix}
\frac{1}{2}(B(t)\gamma_S - 3\pi J_{12}) & 0 & \frac{\pi}{2}(\cos(\theta/2)(J_{13} - J_{23}) + \sin(\theta/2)(J_{13} + J_{23})) \\
0 & \frac{1}{2}(B(t)\gamma_S + \pi J_{12}) & \frac{\pi}{\sqrt{2}}(\cos(\theta/2)(J_{13} + J_{23}) - \sin(\theta/2)(J_{13} - J_{23})) \\
\frac{\pi}{\sqrt{2}}(J_{13} - J_{23}) & \frac{\pi}{\sqrt{2}}(J_{13} + J_{23}) & (\gamma_I - \frac{1}{2}\gamma_S)B(t) + \frac{\pi}{2}(J_{12} - J_{13} - J_{23})
\end{bmatrix},$$  (7)

where the black squares indicate the fact that $[H(t)]_{W_\theta}$ is equal to its transpose.

According to equation 7 the transition frequency between states $|S_0'\beta\rangle$ and $|T_-\alpha\rangle$ in the near-equivalence regime is given by

$$\omega_{ST} = \langle T_-\alpha | H_0 | T_-\alpha \rangle - \langle S_0'\beta | H_0 | S_0'\beta \rangle = B_{\text{bias}}(\gamma_I - \gamma_S) + \frac{\pi}{2}(4J_{12} - J_{13} - J_{23}),$$  (8)
whereas the transition frequency between $|T'_0\beta\rangle$ and $|T'_{-1}\alpha\rangle$ is given by

$$
\omega_{TT} = \langle T'_{-1}\alpha|H_0|T'_{-1}\alpha\rangle - \langle S'_0\beta|H_0|S'_0\beta\rangle
= B_{\text{bias}}(\gamma_I - \gamma_S) - \frac{\pi}{2}(J_{13} + J_{23}).
$$

(9)

Application of a WOLF pulse with $\omega_{\text{WOLF}} = \omega_{ST}$ causes a resonant modulation of the energy difference between states $|S'_0\beta\rangle$ and $|T'_{-1}\alpha\rangle$, but causes an off-resonant modulation of the energy difference between states $|T'_0\beta\rangle$ and $|T'_{-1}\alpha\rangle$ on the order of $\sim J_{12}$. To a first approximation we may neglect the state $|T'_0\beta\rangle$ altogether, and consider a fictitious two-level system evolving under the Hamiltonian $h(t)$

$$
h(t) = \left[ \begin{array}{c}
\langle S'_0\beta|H(t)|S'_0\beta\rangle & \langle S'_0\beta|H(t)|T'_{-1}\alpha\rangle \\
\langle T'_{-1}\alpha|H(t)|S'_0\beta\rangle & \langle T'_{-1}\alpha|H(t)|T'_{-1}\alpha\rangle
\end{array} \right]
= \left[ \begin{array}{c}
\frac{1}{2}(B(t)\gamma_S - 3\pi J_{12}) & \frac{\pi}{\sqrt{2}}(\cos(\theta/2)(J_{13} + J_{23}) + \sin(\theta/2)(J_{13} + J_{23})) \\
\frac{\pi}{\sqrt{2}}(\cos(\theta/2)(J_{13} + J_{23}) + \sin(\theta/2)(J_{13} + J_{23})) & (\gamma_I - \frac{1}{2}\gamma_S)B(t) + \frac{\pi}{2}(J_{12} - J_{13} - J_{23})
\end{array} \right].
$$

(10)

In terms of normalised Pauli matrices $(\sigma_j/2)$ the Hamiltonian $h(t)$ may be expressed as shown below

$$
h(t) = \omega_0\sigma_0 + \omega_x\sigma_x/2 + \omega_z(t)\sigma_z/2,
$$

(11)

with

$$
\omega_0 = \frac{1}{2}\gamma_I(B_{\text{bias}} + B_{\text{WOLF}}^0 \cos(\omega_{ST}t)) - \frac{\pi}{4}(2J_{12} + J_{13} + J_{23}),
\omega_x = \pi\sqrt{2}(\cos(\theta/2)(J_{13} - J_{23}) + \sin(\theta/2)(J_{13} + J_{23})),
\omega_z(t) = -B_{\text{WOLF}}^0(\gamma_I - \gamma_S)\cos(\omega_{ST}t) + B_{\text{bias}}(\gamma_S - \gamma_I) + \frac{\pi}{2}(J_{13} + J_{23} - 4J_{12})
= -B_{\text{WOLF}}^0(\gamma_I - \gamma_S)\cos(\omega_{ST}t) - \omega_{ST}.
$$

(12)

The Pauli coefficient $\omega_x$ may alternatively be expressed as follows

$$
\omega_x = 2\pi\sqrt{J_{13}^2 + J_{23}^2}\sin(\phi + \theta/2),
\phi = \arctan(2(J_{13} + J_{23}, J_{13} - J_{23}),
$$

(13)

where we made use of the following relations

$$
\sqrt{J_{13} + J_{23}\sin(\phi)} = (J_{13} - J_{23})/\sqrt{2},
\sqrt{J_{13} + J_{23}\cos(\phi)} = (J_{13} + J_{23})/\sqrt{2}.
$$

(14)

Within our approximations the $\omega_0$-term introduces an overall phase shift and may be discarded. The WOLF pulse dynamics may now be approximately described within a "jolting" interaction frame. The jolting frame represents a rotating frame with a time-dependent rotation frequency. The time-dependent rotation frequency and the rotation angle $\psi(t)$ are related as follows

$$
\psi(t) = \int_0^t \omega_z(s)ds.
$$

(15)

The jolting frame Hamiltonian $\hat{h}(t)$ is then given by

$$
\hat{h}(t) = \frac{\omega_x}{2}\exp(+i\psi(t)\sigma_z/2)\sigma_x\exp(-i\psi(t)\sigma_z/2)
= \frac{\omega_x}{4}(\exp(+i\psi(t))\sigma_+ + \exp(-i\psi(t))\sigma_-).
$$

(16)

We may expand the jolting frame Hamiltonian as a Fourier series

$$
\hat{h}(t) = \frac{\omega_x}{4} \left( \sum_n J_n(A) \exp(-i(n-1)\omega_{ST}t)\sigma_+ + \sum_n J_{-n}(A) \exp(-i(n+1)\omega_{ST}t)\sigma_- \right),
$$

(17)
where \( J_n(x) \) is the \( n \)’th Bessel function of the first kind and the argument \( A \) is defined as follows

\[
A = (\gamma_I - \gamma_S)B_{\text{WOLF}}^0/\omega_{\text{ST}}.
\]  (18)

For \(|\omega_x/\omega_{\text{ST}}| \ll 1\), which is bounded from above by

\[
|\omega_x/\omega_{\text{ST}}| \leq 2\pi \sqrt{J_{13}^2 + J_{23}^2}/|\omega_{\text{ST}}|,
\]  (19)

we may neglect the time-dependent terms of \( \tilde{h}(t) \). This approach is equivalent to truncating the average Hamiltonian after first order

\[
\tilde{h}(t) \simeq \frac{\omega_x}{4} (J_1(A)\sigma_+ + J_1(A)\sigma_-),
\]

\[
= \omega_x J_1(A)\sigma_x/2.
\]  (20)

The effective nutation frequency of a WOLF pulse is thus given by

\[
\omega_{\text{ST}}^{\text{nut}} = \omega_x J_1(A) = 2\pi \sqrt{J_{13}^2 + J_{23}^2} \sin(\phi + \theta/2)J_1(A).
\]  (21)

II. TECHNICAL DETAILS

A. Samples

The precursor solution for fumarate was prepared by dissolving 100 mM disodium acetylene dicarboxylate, 100 mM sodium sulfite, and 6 mM \([\text{RuCp}^*(\text{MeCN})_3]\text{PF}_6\) (CAS number: 99604-67-8) in \( \text{D}_2\text{O} \), heating to 60°C, and passing through a Millex® 0.22 \( \mu \)m PES filter.

The precursor solution for maleate was prepared by dissolving 100 mM acetylene dicarboxylic acid and 5 mM \([\text{Rh(dppb})(\text{COD})]\text{BF}_4\) (CAS number: 7440-16-6) in methanol-\( d_4 \). All materials and consumables were purchased from Merck.

Under the reaction and solvent conditions used for the experiments, maleate is expected to exist mainly in the form of the protonated singly-charged mono-hydrogen maleate anion, while fumarate is expected to exist as the doubly-charged non-protonated fumarate anion. These protonation states are ignored in the current report, for the sake of simplicity.

B. Equipment

Para-enriched hydrogen was produced by passing hydrogen gas over an iron oxide catalyst packed in 1/4 inch 316L stainless steel tubing cooled by liquid nitrogen. The gas is bubbled through the solutions using a 1/16 inch PEEK capillary tube inserted inside a thin-walled Norell® pressure NMR tube. The Rheodyne MXP injection valves and the Keysight 33500B waveform generator were triggered and controlled by an Arduino Mega 2560 micro-controller board. The waveform generator was connected to the 3 cm wide and 30 cm long solenoid coil of 300 turns placed in the TwinLeaf MS-4 mu-metal shield and used for generating the oscillating magnetic field. The bias field was generated by the built-in Helmholtz coil of the Twinleaf shield, powered by a Keithley 6200 DC current source. A ~200 turn solenoid guide coil was wound around the orifice penetrating the mu-metal shield and continuously driven by a second Keithley 6200 DC current source to produce 6 \( \mu \)T field. The guide coil was used to avoid zero-field crossings during sample transportation.

C. Experimental Procedure

Before each experiment, 250 \( \mu \)L of sample was taken from a 8 mL stock solution and heated in a water bath to a temperature of 90°C. The pressure tube was then quickly placed into the magnetic shield and the hydrogenation experiment was initiated. The bubbling time \( \tau_b \) was set to 10 s and 30 s for experiments involving maleate and fumarate, respectively. After 5 seconds during which polarisation transfer driven by oscillating field is performed, the sample was manually removed from the magnetic shield and inserted into the Oxford 400 MHz magnet equipped with a Bruker Avance Neo spectrometer.
The $^{13}$C free-induction decays were excited by a hard pulse of 14.7 kHz rf amplitude and recorded with 65 k point density at the spectral width of 200 ppm. Additional $^1$H decoupling was used for all experiments. Thermal equilibrium $^{13}$C spectra were recorded at room temperature with recycle delays of 120 s, averaging the signals from 512 and 360 transients for maleate and sodium fumarate, respectively.

**FIG. S1.** Detailed timing diagram for the WEREWOLF procedure. (a) Radiofrequency $^{13}$C pulse applied in high magnetic field at the end of the procedure and NMR signal acquisition. (b) Magnetic field along the z-axis, showing the ambient laboratory field, the reduction in field as the sample is placed in the shield, the bias field $B_{bias}$ during the WOLF pulse, the removal of the sample from the shield and insertion into the high-field NMR magnet. (c) Oscillating WOLF pulse field, applied for a duration $\tau$, with a frequency $\omega_{WOLF}$ and peak amplitude $B^0_{WOLF}$. (d) The total field experienced by the sample is the sum of (b) and (c). Timings for separate phases of the sequence are given below, where bubbling time $\tau_b$ was set to 30 s (for fumarate) and 10 s (for maleate). The time period ($\tau + \tau_s$) was set to 5 s for all experiments.

**D. Frequency optimisation**

As discussed in section I and evident from equation 7 the singlet-triplet transition is effectively driven once the condition $\omega_{WOLF} = \omega_{ST}$ is fulfilled. The experimental optimisation of $\omega_{WOLF}$ is given in the Fig. S2 which was performed at fixed WOLF pulse duration. The experimental data points indicate well defined peaks with FWHM of $\sim 0.4$ Hz and $\sim 6$ Hz for fumarate and maleate, respectively. The match between numerically simulated profiles and the data suggest that field inhomogeneity is negligible. The discrepancy in resonance width could be associated with the different number of magnetic field oscillations required to reach maximum signal at $\tau = \pi/(2\omega_{int})$. 
FIG. S2. Hyperpolarised fumarate (a) and maleate (b) $^{13}$C signal intensity as a function of $\omega_{\text{WOLF}}$ at fixed duration $\tau$ equal to 50 and 9 magnetic field oscillations during the WOLF pulse for fumarate and maleate, respectively. Black dots depict experimental data whereas grey lines represent numerical simulations using the spin-spin coupling parameters found in the manuscript.