Three-spin solid effect and the spin diffusion barrier in amorphous solids

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INTRODUCTION

Microwave-driven dynamic nuclear polarization (DNP) (1, 2) is a well-established method used to enhance nuclear magnetic resonance (NMR) signal intensities by transferring polarization from unpaired electrons to nearby nuclei. Subsequently, this polarization is transferred to more distant nuclei and polarizes the entire sample via spin diffusion (3). There are four different mechanisms that use continuous wave microwave irradiation to drive the polarization transfer in DNP, namely, the Overhauser effect, cross effect (CE), solid effect (SE), and thermal mixing (4–7). In general, these mechanisms yield large signal enhancements (≥100) leading to experimental time savings of ≥10^4. However, in an amorphous, glassy, glycerol-water matrix, which is a medium frequently used for DNP, the mechanism by which the electron polarization moves from the polarizing agent to the nuclei of the solvent or target molecule is not known. Here, we use our recent experimental observation and theoretical analysis of the three-spin solid effect (TSSE), together with 1H electron nuclear double-resonance (1H ENDOR) and ELDOR-detected NMR (EDNMR) spectra, to address this question. We find that, for the case of trityl radical dispersed in glycerol-water solvents, the 1H's polarized in the initial step of DNP are less than a few angstroms from the electron and reside on the trityl molecule. Furthermore, 1H ENDOR experiments indicate that the electron polarization is first transferred to the intramolecular 1H's on the trityl radical and then via spin diffusion to glycerol molecules in intimate contact with the trityl.

The SE mechanism, on which this paper is largely based, was first discussed theoretically by Jeffries (8) and later demonstrated experimentally by Abragam and Proctor (4). In an SE experiment, microwave irradiation is applied at an electron offset frequency that matches the nuclear Larmor frequency (ΩI = ±ωI) to drive the polarization transfer via either a double-quantum (DQ) or a zero-quantum (ZQ) transition (Fig. 1A). Although these transitions are formally forbidden, they become weakly allowed because of the mixing of nuclear Zeeman eigenstates via the $S, I^z$ terms in the dipole Hamiltonian as illustrated in Fig. 1 (9–13). In 1976, there was a cursory mention by de Boer et al. (14, 15) of the observation of a higher-order SE in 2H spectra recorded at 0.7 K and 2.5 T with an enhancement of ~3 at twice the nuclear Larmor frequency (ΩI = ±2ωI). Although the effect was not analyzed, it was suggested to be due to a three-spin system composed of an electron and two nuclei, i.e., TSSE. Similarly, the matching conditions at (ΩI = ±ωI) are attributed to the excitation of the single-quantum (SQ) or triple-quantum (TQ) transition (Fig. 1A) present in the three-spin system. The weak enhancement observed was not surprising given that the forbidden transitions exploited at the normal SE condition are only weakly allowed, and thus, exciting the highly forbidden TSSE transition is expected to be more challenging. More recently, Smith et al. (16) attempted to reinvestigate the TSSE, but the enhancement was not directly observable. In addition, a similar multinuclear effect, 1H–13C heteronuclear CE, was demonstrated at 3.4 T and 6.5 K (17). However, the actual enhancement factor was not reported because of the inability to observe the thermal equilibrium signal. Here, we report an enhancement factor ε ~ 170 (vide infra) at the TSSE conditions using trityl radical dispersed in a glassy glycerol-water matrix at 0.35 T and 80 K. To our knowledge, this is the largest enhancement ever observed for such an effect. We have analyzed the TSSE theoretically and by numerical simulations, which show that the effect is very sensitive to the electron-nuclear distances r. Hence, we exploit this feature to probe the structure and chemical environment around the radical and to provide insight into the DNP polarization pathway involving the spin diffusion mechanism (3, 18).

As mentioned earlier, the spin diffusion process plays an essential role in transferring the polarization from the DNP-enhanced near nuclei to the bulk nuclei. However, the nuclei neighboring the polarizing agent are generally thought to experience a large local field originating from the hyperfine interaction, and this induces a shift in the precession frequency away from the bulk resonances and thereby quenches the spin diffusion mechanism. Moreover, the random modulation of the hyperfine interactions could lead to fast relaxation rates for the near nuclei and hence relaxes the hyperpolarized signal. The
near nuclei in this regime are often referred to as the “core” nuclei, which reside within the spin diffusion barrier (Fig. 1B) (19–21), and their polarization has a limited contribution to the bulk signal. There have been several attempts to quantify the size of this barrier with examples of low-temperature studies of single crystals doped with paramagnetic ions (20, 22); however, a broad range of values ranging from 3 to 17 Å are reported in the literature (20, 22–28). Given the ongoing renaissance in DNP using bulky organic radicals in amorphous glassy matrices, as opposed to paramagnetic metal ions in single crystals, it is important to determine the size of the spin diffusion barrier on an amorphous frozen solution such as OX063 in glycerol/water. In this contribution, using several independent approaches such as using trityl radicals with deuterated side chains and solvents with various degree of deuteration, we show that the limit on the size of the spin diffusion barrier is <6 Å for trityl radical in an amorphous glycerol-water matrix. Furthermore, a comparison of $^1$H ENDOR spectra of $^2$H and $^1$H glycerol reveals that the trityl radicals form a cluster with the glycerol molecules, shielding themselves from the water molecules. Thus, the initial step in the polarization transfer is to $^1$H’s on glycerol. These results provide important information on the spin diffusion pathway and aid the design of DNP polarizing agents for applications in a number of different areas.

Theory

We consider a three-spin $e^{-1}$H-$^1$H system ($\psi = |ijk\rangle$), where the quantum numbers $i$, $j$, and $k$ refer to the spin states of the electron, nucleus 1, and nucleus 2, respectively. The general Hamiltonian of such a system in the electron rotating frame is given by

$$\hat{H} = \Omega_0 \hat{S}_z - \omega_0 (\hat{I}_{1z} + \hat{I}_{2z}) + \hat{S}_z (A_1 \hat{I}_{1z} + A_2 \hat{I}_{2z}) + \hat{S}_z (B_1 \hat{I}_{1z} + B_2 \hat{I}_{2z})$$

where $A$ and $B$ are the secular and nonsecular components of the hyperfine interaction, respectively, and $\Omega_0$ is the microwave offset frequency. The Hamiltonian $\hat{H}$ can be transformed to a diagonalized form $\hat{H}^A$ by

$$\hat{H}^A = \hat{U} \hat{H} \hat{U}^{-1}$$

where $\hat{U} = \exp \left(-i \sum_{k=1,2} (\eta_{k\alpha} \hat{S}_z \hat{I}_{ky} + \eta_{k\beta} \hat{S}_y \hat{I}_{ky}) \right)$

and angles $\eta_{k\alpha/\beta}$ are given by

$$\eta_{k\alpha} = \tan^{-1} \frac{-B_k}{A_k - 2\omega_0}; \quad \eta_{k\beta} = \tan^{-1} \frac{-B_k}{A_k + 2\omega_0}$$

where $\hat{I}$ represents the identity operator. Following that, the eigenvalues and henceforth the matching conditions for the SQ $|\alpha\beta\gamma\rangle \leftrightarrow |\beta\alpha\gamma\rangle$ and TQ $|\alpha\alpha\gamma\rangle \leftrightarrow |\beta\beta\gamma\rangle$ transitions, which are responsible for the TSSE, can be determined from the diagonalized Hamiltonian $\hat{H}^A$ (Eq. 2) using Mathematica (Wolfram Research)

$$\Omega_0 = \pm \sum_{k=1,2} \left( \sqrt{B_k^2 + (A_k + 2\omega_0)^2} + \sqrt{B_k^2 + (A_k - 2\omega_0)^2} \right) / 4$$

Following that, the transition amplitudes of the TQ and SQ transitions (see the Supplementary Materials for details of the derivation) are given by

$$a_{ij} = \langle \psi^A | \hat{U} \hat{O}_{ij} \hat{S}_z \hat{U}^{-1} | \psi^A \rangle$$

$$a_{18} = a_{45} = \frac{-\omega_{15}}{8} \left( e^{i(\eta_{1\alpha} + \eta_{1\beta} + \eta_{1\gamma})/2} - e^{-i(\eta_{1\alpha} + \eta_{1\beta} + \eta_{1\gamma})/2} \right) (e^{-i\eta_{1\alpha}} - e^{-i\eta_{1\beta}}) (e^{-i\eta_{1\alpha}} - e^{-i\eta_{1\beta}})$$

$$\approx \frac{B_1 B_2 \omega_{15}}{8\omega_{01}} \text{ if } A_k, B_k \ll \omega_{01} \text{ and } \omega_{15} \ll \omega_{01}$$
where \( \psi^A \) are the eigenstates in \( \hat{H}^A \). Typically, \( A_3/2\pi \approx 1 \) MHz and \( \omega_{1S}/2\pi \approx 15 \) MHz, so the inequalities are satisfied. Note that the TSSE transition amplitudes (Eq. 5) are proportional to \( B^2 \) or \( r^6 \) where \( r \) is the electron-nucleus distance assuming \( B_1 = B_2 \). Moreover, the transition amplitudes \( a_{18}(a_{45}) \) (Eq. 5) show that the highly forbidden TQ (SQ) transitions depend on the product of the two electron-nucleus dipolar couplings and that the nuclear-nuclear coupling is not required, as confirmed by numerical simulations. Similarly, the transition amplitudes of a normal two-spin SE can be derived from \( \hat{H}^A \) (Eq. 2) and shown to be

\[
a_{35} = -a_{17} = \frac{B\omega_{1S}}{4\omega_{01}}
\]

which, assuming \( B_1 = B_2 \), is \( \sim 2\omega_{01}/B \) times larger than those of the TSSE (Eq. 5). Following that, the transition probabilities can be determined by applying Fermi’s golden rule

\[
P_{ij} = |a_{ij}|^2
\]

\[
P_{17} = P_{35} = \frac{B^2\omega_{1S}^2}{16\omega_{01}^4}
\]

\[
P_{18} = P_{45} = \frac{B_1^2B_2^2\omega_{1S}^4}{64\omega_{01}^8}
\]

which yields the well-known result that the transition probability of the SE \( P_{17} \) (or \( P_{35} \)) is proportional to \( \omega_{01}^{-2} \). Note that the TSSE transition probability \( P_{18} \) (or \( P_{45} \)) has a field dependence of \( \omega_{1S}^{-4} \). Thus, it is not surprising that the TSSE enhancement is small or not easily observable at higher fields (15, 16). Further discussion on the relevance of Fermi’s golden rule, Rabi frequency \( \omega_{1S} \), and relaxation effect on the DNP enhancement can be found in the literature for interested readers (10).

RESULTS AND DISCUSSION

The field profiles in Fig. 2A show that the bulk \(^1\text{H} \) nuclei are hyperpolarized at the SE and TSSE conditions at \( \Omega_S = \pm 15 \) MHz and \( \Omega_S = \pm 30 \) MHz, respectively. The experimental observations agree well with the predicted matching conditions (Eq. 4) and the energy level diagram (Fig. 1A). The enhancement factors are \( \varepsilon \approx \pm 220 \) for OX063 trityl at the SE conditions and \( \varepsilon \approx 100 \) for the TSSE, using a Rabi frequency of \( \omega_{1S}/2\pi = 2 \) MHz and a polarization time of \( \tau = 8 \) s (see fig. S1). The TSSE enhancement can be further optimized to obtain \( \varepsilon \approx 170 \) (vide infra) by using a longer \( \tau \) and higher \( \omega_{1S} \).

Effect of solvent deuteration on the TSSE

The theoretical analysis of the TSSE implies that the enhancement factor is sensitive to the electron-nuclei distance \( r \), and this has enabled us to examine the DNP polarization pathway from the radical to the bulk \(^1\text{H} \) nuclei. Before directly investigating that issue, it is important to justify that the \(^1\text{H} \)’s involved in the TSSE originate from the intramolecular protons on the radical, as opposed to the solvent. We recorded field profiles using similar conditions but with solvents of different \(^1\text{H} \) concentrations (Fig. 2B), i.e., double or half of the \( \text{H}_2\text{O} \) volume ratio in “DNP juice” \( \text{d}_4\text{-glycerol:D}_2\text{O:H}_2\text{O}, 60:30:10 \) by volume. In principle, altering the \(^1\text{H} \) concentration directly influences the DNP enhancement because it changes the average number of

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Fig. 2. EPR spectrum, DNP field profiles, and EDNMR spectra at 0.35 T. SE-DNP field profiles (A to C) for (A) various trityl radicals, (B) trityl OX063 with solvents of different \( \text{H}_2\text{O} \) ratios, and (C) simulations with different distances \( r_{se} \). An electron paramagnetic resonance (EPR) spectrum of OX063 (magenta) is also shown in (A) as a reference. The other trityl radicals have similar spectra and therefore are not shown. The field profiles in (B) are all normalized with the enhancement obtained at the DQ-SE (\( \Omega_S = -15 \) MHz) condition. (D) Normalized ELDOR (electron-electron double resonance)--detected NMR (EDNMR) spectra with an inset in an expanded view were acquired using a saturating pulse of 3 ms. All field profiles were obtained by changing the microwave frequency while keeping the magnetic field constant, which could explain the slight asymmetry observed in the field profiles, as the Rabi frequency might be different at different microwave frequencies.
The good agreement between numerical simulations, experimental results, and analytical theory supports our hypothesis that the polarization in the TSSE is mediated via the protons on the radical, which then contributed to the observed bulk 1H signal via spin diffusion. In other words, the intramolecular protons, such as the methylene –CH2 groups in OX063 or the methyl –CH3 groups in Finland trityl radical, must be outside the spin diffusion barrier or else we would not be able to enhance the polarization of the bulk protons at the TSSE conditions. As the average distances of the methyl protons are reported to be 5.6 ± 0.2 Å (31) and the methylene protons are ~6.0 Å [from the density functional theory (DFT) calculations explained in the Supplementary Materials], we conclude that the radius of the spin diffusion barrier is <6 Å. This result is in good agreement with the size of a spin diffusion barrier surrounding the vanadyl complex, which was found to be between 4 and 6.6 Å using electron paramagnetic resonance (EPR) spectroscopy (28).

**Effect of deuterating the trityl radicals on the TSSE**

To further support our assertion that the TSSE is mediated via the intramolecular protons, we repeated the experiments on various trityl radicals (Fig. 1B) under the same conditions. The experimental results show that the TSSE enhancements decrease by ~50% (Fig. 2A) when the closest 24 methylene –CH2 groups in OX063 are deuterated, while the change in normal SE enhancements is smaller, i.e., 18% drop in e.

We presume that the TSSE enhancements are not fully suppressed because of the remaining protonated methylene groups farther from the electron. On the other hand, similar experiments performed on the Finland trityl show that the TSSE e quenched by ~90%, i.e., from ~100 to ~9 ± 3 (slightly above noise level) when perdeuterated d36-Finland is used. Here, the absence of any remaining intramolecular protons renders the TSSE nearly unobservable. The experimental findings on these two different classes of radicals are in good agreement with our earlier assertion that the intramolecular protons are crucial in mediating the TSSE, whose transition amplitude has a ~B2 dependence (Eq. 5) and therefore becomes inefficient if the closest protons are farther away (d36-OX063 and d36-Finland).

In addition, we performed numerical simulations on a simple three-spin model. To represent the large number of proton spins (up to 48 protons) on the radical with only two protons, we have adopted the notion of effective distance, r_eff (29–31), with values of r_eff = 3.8, 4.4, and 5.5 Å to model the OX063, d36-TOX063, and d36-Finland trityl radicals, respectively. We neglected the protonated Finland trityl in the simulations because the methyl group dynamics might affect the DNP enhancement and are ill posed in our simplified description. The value of r_eff for the d36-Finland was adopted from the estimated distances of the solvent protons (31, 32), while the justification of other r_eff values was explained in the numerical simulation section (vide supra). The numerical results (Fig. 2C) show that e is in fact more sensitive to r_eff in the TSSE than in the normal SE, i.e., e at Q = ±30 MHz is attenuated by ~50 and 90% (Table 1) when the value of r_eff increases from 3.8 Å to 4.4 and 5.5 Å, respectively. We note that the TSSE lines are narrower in the simulation than in the experimental data, and it could be that a three-spin system is insufficient to fully represent a trityl molecule, i.e., more protons with a distribution of hyperfine couplings are required. Another consideration is that the relaxation parameters of the nuclei (T1n and T2n) might not be the same for all nuclei, and there might be a distribution of values for these parameters depending on their distances from the electron. These effects are, however, difficult to incorporate into numerical simulations.

| Table 1. Values of the parameter r_eff used and the enhancements factors extracted from Fig. 4. The renormalized e_TSE values reported are normalized with respect to the values obtained for OX063 in the first column. |
|---|---|---|---|
|  | OX063 | d36-TOX063 | d36-Finland |
| r_eff (Å) | 5.98 | 6.96 | 5.5 |
| Actual exp | 3.66 | 4.6 | 5.5 |
| Expt e_TSE (normalized) | 93/93 | 40/93 | 11/93 |
| Sim e_TSE (renormalized) | 0.498/1 | 0.218 | 0.059 |

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splitting, and this value is assigned to the parallel principal value of a purely dipolar interaction between the proximate solvent $^1$H and the radical. For the case of DNP juice, the full breadth of the extreme ends of the ENDOR line is $\sim 0.9$ MHz, which yields an estimated lower limit of the $e^{-} - ^1$H distance to be $\sim 5.6$ Å. Note that this is a qualitative analysis and resembles only a tiny fraction of the solvent $^1$H's close to the electron. The result shows that, in the optimal (but unlikely) scenario, most solvent protons are farther away from the radical than the electron-CH$_3$ distances. The results agree well with our hypothesis that the intramolecular protons on the radical are the main contributors to the TSSE. Besides that finding, the weak hyperfine interaction observed between the water molecules and the electron also explains the similar field profiles when the solvent $^1$H ratio is altered (Fig. 2B).

Note that we have only quoted an upper limit for the size of the barrier. To more accurately probe the size of the barrier, i.e., determining the lower limit, one would need to synthesize an array of stable radicals with different electron-nuclear distances with an option of $^2$H isotope labeling. The approach is beyond the scope of this work. Moreover, the spin diffusion barrier is merely a classical model. In reality, the barrier should be orientation dependent rather than spherical because of the anisotropic nature of dipolar couplings (19). Last, the rate of spin diffusion should experience a gradual change near the barrier rather than an abrupt jump as proposed in the model.

In addition, we have also performed the ELDOR (electron-electron double resonance)–detected NMR (EDNMR) (33) experiments (Fig. 2D) to cross-verify the DNP field profiles. The experiment is similar to SE-DNP except that the electron polarization signal is monitored instead of the bulk $^1$H. Since the total polarization is conserved, the gain in NMR polarization in SE-DNP must originate from the loss of electron polarization (16). Thus, the loss of electron polarization due to saturation of the forbidden transitions can provide complementary information to the DNP efficiency. For instance, we observed $\varepsilon \sim 93, 94, 40,$ and 11 in DNP (Fig. 2A) for the OX063, Finland, $d_{36}$-OX063, and perdeuterated $d_{34}$-Finland trityls, respectively. These enhancement numbers in ratio, i.e., $93:94:40:11$ or approximately 9:9:4:1, correlate well to the $\sim 11, 8, 5,$ and 1% of the electron polarization attenuated at $\Omega_0 = 30$ MHz in EDNMR. Another advantage of performing EDNMR experiment is that it can be used to detect the presence of nearby nuclei within the spin diffusion barrier, which act as a sink for electron polarization. These nearby nuclei are, however, not easily observable in the DNP experiment. Nevertheless, we notice that the saturation factor at $\Omega_0 = \pm 15$ MHz has little relevance to the SE $\varepsilon$, and it is likely that the microwave field is reaching a regime where the nearby nuclei are polarized by DNP more rapidly than the bulk nuclei are polarized by spin diffusion (vide infra).

**Buildup curve and power dependence of the TSSE**

We emphasize that the transition probability $P_{ij}$ directly influences, but does not dictate, the nominal enhancement factor. In particular, a similar enhancement factor can be achieved by exploiting a longer mixing time for experiments using a weaker $\omega_{1S}$ in near-ideal cases (34, 35), for instance, when the DNP mechanism is sufficiently efficient relative to the rate of spin diffusion and the relaxation constants. This is supported by the measured enhancements of $\varepsilon \sim 175$ to 250 (36) obtained using the NOVEL (nuclear orientation via electron spin locking) sequence, which theoretically has a higher transition probability but similar $\varepsilon$ compared to SE. Figure 4 shows the dependence of the enhancement $\varepsilon$ on the Rabi frequency $\omega_{1S}$ and the mixing time $\tau$. At $\omega_{1S}/2\pi \sim 0.2$ MHz, the ratio of the enhancement from the TSSE to the two-spin SE condition is only $\varepsilon_{TSSE}/\varepsilon_{SE} = 17/185 \sim 0.09$ and continues to increase as $\omega_{1S}$ increases. This is as expected since a larger $\omega_{1S}$ is required to saturate the highly forbidden TSSE condition. Both enhancement factors begin to saturate at $\sim 2$ MHz, and the ratio becomes $\varepsilon_{TSSE}/\varepsilon_{SE} = 168/284 \sim 0.6$, which is evidently higher than $P_{1S}/P_{1S} = (B^2)/(4\omega_0^2) \sim 10^{-3}$ (Eq. 7). This shows that the concept of directly correlating transition probability $P_{ij}$ to the actual enhancement $\varepsilon$, as it is usually adopted in the literature, does not necessarily suffice in all cases. Nevertheless, one can show theoretically that this notion is applicable in characterizing the initial polarization buildup at a short time $t$ such that $\sqrt{P_{ij} t} \ll 1$. We believe that the ratio $\varepsilon_{TSSE}/\varepsilon_{SE}$ reaches only 0.6 instead of unity because it is still fundamentally limited by the proton relaxation rate. The polarization buildup curve (Fig. 4B) shows that the increments in the DNP enhancement for both conditions are marginal past $T_{1S} \sim 13$ s. The results shown here have important implications to the observation of TSSE at higher fields (16), which was deemed difficult because of the unfavorable scaling of $P_{TSSE} \sim \omega_0^{-1}$ (Eq. 7). Nevertheless, we hypothesize that moderate

![Fig. 3. $^1$H ENDOR spectra using different solvent conditions at 0.35 T. Normalized $^1$H ENDOR spectra at 80 K recorded on 0.5 mM $d_{26}$-Finland trityl in 60:40 by volume glycerol/water mixtures, with either deuterated or protonated components. The mixtures are “DNP juice” ($d_2$-glycerol/D$_2$O/H$_2$O, 60:30:10 by volume; black), $^1$H glycerol with 99.9% D$_2$O (blue), or 99.9% H$_2$O (red).](image-url)
enhancement can be achieved by exploiting higher nutation frequency \( \omega_{1S} \) and/or lower temperatures \((14, 15)\).

**MATERIALS AND METHODS**

**Sample preparation**

We performed DNP and EPR studies on four different trityl radicals, namely, OX063, deuterated d_{36-OX063}, Finland \((37)\), and perdeuterated d_{36-Finland} \((Fig. 1B)\). The trityl radicals were obtained from GE Healthcare \((Amersham, UK)\). Samples of 5 mM trityl dispersed in a mixture of d_{6-glycerol}:D_{2}O:H_{2}O \((60:30:10 by volume)\), also known as DNP juice, were used in all DNP experiments except for the dilution analysis \((Fig. 2B)\).

**DNP-NMR and EPR spectroscopy**

Using instrumentation similar to that described previously \((35, 38, 39)\), we performed all experiments at a temperature of \( T = 80 \text{ K} \) and \( B_0 = 0.35 \text{ T} \). The presaturation of the targeted \( ^1\text{H} \) signal intensity, with microwaves turned on, scaled with the same integrated signal without microwave irradiation at full \( ^1\text{H} \) thermal equilibrium, extrapolated from the measured signal at ~3\( T_{1\text{H}} \). The EPR spectra were obtained by using spin echo–detected spectra in a field-swept experiment. The Mims ENDOR experiments, i.e., \( [\pi/2-d-\tau/2-\text{RF}(T_{\text{RF}})-\pi/2-d-\text{echo}] \), were performed using a Bruker DICE-II ENDOR system with a 250-W radio frequency (RF) amplifier. The delay \( d = 300 \text{ ns} \) was chosen so that the blind spots occur at integer multiples of \( 1/\text{RF} \) – 3.33 MHz, which are much larger than the largest hyperfine coupling expected in trityl. RF pulses \((25 \text{ W})\) and \( T_{\text{RF}} = 160 \text{ ms} \) were used to excite the allowed NMR transitions. The RF sweep was performed using a stochastic mode. The EDNMR experiments were performed with a high-turning angle pulse of 3 ms with a Rabi frequency of 2 MHz at 80 K.

**Numerical simulations**

The numerical simulations were performed using a custom-built simulation package \((40)\) written in MATLAB \((MathWorks)\). Relaxation effect was incorporated in the program using relaxation superoperators in Liouville space \((41)\). Relaxation times of \( T_{1\text{H}} = 0.6 \text{ ms} \) and \( T_{2\text{H}} = 5 \text{ ms} \) for the electron and of \( T_{1\text{N}} = 13 \text{ s} \) and \( T_{2\text{N}} = 1 \text{ ms} \) for both \(^1\text{H}\) nuclei were used in the simulations. We used \( \omega_{1S/2\text{e}} = 2 \text{ MHz} \), \( \tau = 16 \text{ ms} \), and 100 different powder orientations chosen using the zero correlation window scheme in all simulations \((42)\). Further details of the simulation package can be found in the Supplementary Materials and in our recently published work \((40)\). We determined the effective electron–nuclei distance by computing \( r_{\text{eff}} = \langle N/2 \rangle^{1/2}/\langle R \rangle \) \((Table 1)\), where \( \langle R \rangle \) is the average electron–nuclei distances of the closest nuclei in the first shell considered for our simulation and \( N \) is the number of nuclei. Note that the factor 2 was included because two protons were considered in the numerical simulations. The values of \( \langle R \rangle \) for the OX063 radical were determined from the DFT calculations \((see the Supplementary Materials)\), and we used an intermediate value of 5.5 Å for the case of solvent protons \((for d_{36-Finland})\), which were reported to be in the range of 4.8 to 5.8 Å away from the radical \((31, 32, 43)\).

**CONCLUSION AND OUTLOOK**

We have demonstrated in this article the observation of an enhancement \( \varepsilon = 170 \) for the TSSE for the trityl radical at 0.35 T and 80 K, which is the largest value reported to date for this effect. The experimental results are consistent with the theoretical analysis and numerical simulations, i.e., a three-spin electron–nuclei–nuclei system is required to explain the experimental observation. Furthermore, the fact that the transition amplitude of the TSSE is proportional to \( r^{-6} \) allows us to use it as a tool to provide structural information of the radical, as well as the spin diffusion pathway during the DNP process. For instance, our study showed that the TSSE-DNP is mediated via the intramolecular \(^1\text{H}\) rather than the solvent \(^1\text{H}\). Following that result, it permits us to probe the size of the spin diffusion barrier, which arises because of strong hyperfine interactions with the nearby nuclei. Our results have shown that the size of the barrier is ~60 Å for the important organic radical trityl in a glycerol–water matrix. This represents the first measurement of the size of the spin diffusion barrier for a bulky organic radical in amorphous solids. In addition, the \(^1\text{H}\) ENDOR spectra are consistent with the idea that the glycerol molecules are in close proximity around the radical, shielding the hydrophobic part from the water molecules. Furthermore, this independent EPR data indirectly supports our results on the size of the spin diffusion barrier obtained with TSSE-DNP. By combining the results from ENDOR and TSSE-DNP, we are able to establish the DNP polarization pathways starting from the unpaired electron to the bulk nuclei. For this study on the trityl radicals in the glycerol–water mixture, the polarization from the unpaired electron is transferred first to the intramolecular \(^1\text{H}\) via direct DNP \((Fig. 1B)\) and then relayed to the bulk solvent via the glycerol molecules mediated by the spin diffusion mechanism. Our study has successfully elucidated one of the polarization transfer pathways in DNP. An understanding of the size of the spin diffusion barrier and the chemical environment surrounding the polarizing agent in amorphous matrices provides information to constrain the design of new radicals with optimized performance in DNP applications. For instance, the fact that there are no protons <6 Å distance from the central carbon atom (or within the spin diffusion barrier) of trityl, which could act like a polarization sink leading to inefficient DNP transfer, could explain why trityl is one of the more efficient DNP polarizing agents. Last, we note that, among the stable free radicals currently in use as polarizing agents, trityl is unique in that the electron is largely localized \((spin density \sim 0.75)\) \((32)\) on the central carbon atom. This is in contrast to 1,3-bis(diphenylene)-2-phenylallyl \((BDPA)\), where the TSSE was first observed, and the electron is delocalized over the diphenylene rings. This results in large isotropic hyperfine interactions with nearby protons that are revealed by the EPR spectrum in a solution state \((44)\). A third case is bis-nitroxides where the two unpaired electrons are localized on the N–O moieties. Thus, it is of interest to design experiments to examine the size of the diffusion barrier in these polarizing agents and to determine whether it is similar to or different from our current findings for the trityl radical.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/7/eaax2743/DC1

Section S1. Details of the simulation package
Section S2. DFT calculations on trityl OX063
Fig. S2. DFT-optimized structure of trityl OX063.

Fig. S1. Schematic drawing of the DNP pulse sequence.


36. Finlad trityl.

