Electronic Supplementary Information

The efficiency of DPPH as a polarising agent for DNP-NMR spectroscopy

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I. W-band ESR spectra of BDPA, DPPH, and 4-oxo-TEMPO at 100 K

As mentioned in the main text, the ESR properties of the free radicals are crucial elements to the DNP process. As such, we have measured the ESR spectra of the free radicals discussed in this work. Fig. S1 displays the W-band ESR spectra of (a) 1,3-bisdiphenylene-2-phenylallyl (BDPA; 20 mM in 1:1 v/v sulfolane:DMSO), (b) 2,2-diphenyl-1-pycrylhydrazyl (DPPH; 20 mM in 1:1 v/v sulfolane:DMSO), and (c) 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (4-oxo-TEMPO; 15 mM in 1:1 glycerol:water) measured at 100 K. It is evident that the DPPH base to base linewidth $D$ (290 MHz) is intermediate between that of BDPA (62 MHz) and 4-oxo-TEMPO (465 MHz). The ESR spectra of the N-centered DPPH and the nitroxide-based 4-oxo-TEMPO show similar features due to the hyperfine interaction of the paramagnetic electron with the quadrupolar $^{14}$N nuclei. These data at 100 K should reflect the same features of the ESR spectra at DNP conditions (1.4 K).

Fig. S1 W-band ESR spectra of (a) BDPA (20 mM in 1:1 v/v sulfolane:DMSO), (b) DPPH (20 mM in 1:1 v/v sulfolane:DMSO), and (c) 4-oxo-TEMPO (15 mM in 1:1 v/v glycerol:water) measured at 100 K. The insets are the corresponding structures of the free radicals.
II. DPPH electronic magnetisation recovery fitting curves

Fig. S2 shows the W-band electronic magnetisation recovery curves for 20 mM DPPH at (a) 100 K and (b) 5 K. These relaxation data were fitted to different exponential recovery equations to extract the electron spin-lattice relaxation time $T_{1e}$: (i) Single exponential equation $M_z(t)=M_0[1-\exp(-t/T_{1e})]$, (ii) stretched exponential equation $M_z(t)=M_0[1-\exp(-(t/T_{1e})^\beta)]$ where the exponent $\beta$ is the “stretching” parameter, and (iii) double exponential equation $M_z(t)=M_0[1-\exp(-t/T_{1e})-\exp(-t/T_{cr})]$ where the longer relaxation component gives the $T_{1e}$ value and the shorter component $T_{cr}$ is ascribed to cross relaxation effects. At 100 K, the stretched ($T_{1e}=0.7783$ ms with $\beta=0.64$) and double ($T_{1e}=1.87$ ms with $T_{cr}=0.31$ ms) exponential recovery equations give excellent fits with the data in Fig. S2a whereas the single exponential equation gave a sufficiently good fit with $T_{1e}=1.13$ ms. However at 5 K, it is evident that a single exponential equation is incompatible with the data. The stretched exponential equations yielded a good fit with the 5 K data which $T_{1e}=21$ ms but the stretching parameter dropped from 0.64 to 0.24. The double exponential equation gave the best fit to the 5 K data with $T_{1e}=30.1$ ms and $T_{cr}=0.43$ ms. This type of relaxation behaviour (bi-exponential electronic magnetisation recovery curve) was also observed in TEMPO (40 mM) at high fields where cross relaxation effects become evident at such relatively high concentration of free radicals.2

Fig S2 Recovery curve of the electron magnetisation of 20 mM DPPH in 1:1 sulfolane:DMSO taken at W-band and 5 K. The dashed lines are fits to the data.
III. W-band electronic relaxation of BDPA

The W-band electronic magnetisation recovery curves of BDPA (20 mM in 1:1 v/v sulfolane:DMSO), shown in Fig. S3, were traced by saturation recovery technique. Similar to the fitting procedure used for DPPH, a single exponential recovery equation did not yield an appropriate fit especially at low temperatures; a double exponential recovery equation was found to be more appropriate where the longer component gives the $T_{1e}$ value as discussed before. Since the concentration and glassing conditions were the same for both BDPA and DPPH ESR samples, a direct comparison can be made. BDPA has longer electronic $T_{1e}$'s than DPPH, with $T_{1e}=21.8$ ms at 100 K and $T_{1e}=176$ ms at 5 K as shown in Fig. S3; DPPH $T_{1e}=1.87$ ms at 100 K and $T_{1e}=30.1$ ms at 5 K as illustrated in Fig. S2. This can be qualitatively ascribed to the less g-anisotropy and hyperfine interaction relaxation contribution in BDPA than DPPH.

![Fig. S3 W-band electronic magnetisation recovery curves of 20 mM BDPA in 1:1 v/v sulfolane:DMSO measured at (a) 100 K and (b) 5 K. The dashed lines are fits to a double exponential recovery equation where the longer component as described previously.](image-url)
IV. Optimisation of DPPH concentration for DNP

Fig. S4a shows the representative $^{13}$C polarisation buildup curves of 1:1 [1-$^{13}$C]ethyl pyruvate:sulfolane doped with the optimum DPPH concentration (20 mM or 40 mM) for DNP at 3.35 T and 1.4 K. A maximum polarisation of ~5 % was achieved for the optimum DPPH-doped sample, whereas the same sample doped with BDPA yielded a $^{13}$C polarisation of 11 %. As mentioned in the main text, the higher polarisation achieved with BDPA is a consequence of its small ESR linewidth $D$, which implies that a lower spin temperature of the electron dipolar system can be achieved with DNP via thermal mixing, thus resulting in “colder” spin temperature (higher polarisation) of the nuclear Zeeman system.1,3 Fig. S4b displays the plot of the dependence of the $^{13}$C polarisation with DPPH concentration where it can be seen that the polarisation is maximum in the concentration range 20-40 mM.

**Fig. S4** (a) Polarisation buildup curves of 1:1 [1-$^{13}$C]ethyl acetate:sulfolane doped with the optimum concentrations of DPPH (20 mM or 40 mM) and BDPA (40 mM). (b) Summary of the dependence of $^{13}$C polarisation with DPPH concentration. These data were taken at 3.35 T and 1.4 K.
V. $^{13}$C microwave DNP spectra of samples doped with DPPH and DPPH-derivative

Fig. S5 shows the microwave DNP spectra of 1:1 v/v [1-$^{13}$C]ethyl acetate:sulfolane doped with a) 20 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) and b) 20 mM of a DPPH derivative, 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl. These data were taken in the HyperSense at 3.35 T and 1.4 K with a 100 mW microwave source. The features of the $^{13}$C DNP spectra are nearly identical for both free radicals; a slight shift up in frequency (downfield shift) was observed for the DPPH-derivative due to the chemical modifications made in the phenyl groups.

![Fig. S5 Structures of (a) 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (b) a DPPH derivative 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl. (c) $^{13}$C microwave DNP spectra of 1:1 v/v [1-$^{13}$C]ethyl acetate:sulfolane doped with DPPH (20 mM) and a DPPH derivative (20 mM; see structure in Figure 1b). These data were taken at 3.35 T and 1.4 K with a 100 mW microwave source.](image-url)
VI. $^{15}$N $T_1$ decay curves of hyperpolarised pentaerythrityl tetraazide

In fast dissolution DNP-NMR, long spin-lattice relaxation time $T_1$ translates to long lifetime of the hyperpolarised state. Fig. S6 shows the decay of the hyperpolarised $^{15}$N NMR signals emanating from the CH$_2$-linked, central, and terminal $^{15}$N (natural abundance) nuclei of pentaerythrityl tetraazide in methanol solution. These data were collected at 9.4 T and 298 K. The decay of the hyperpolarised signal was monitored by applying a small rf detection pulse with flip angle $\theta_{\text{flip}}=5^\circ$ every time interval $\text{TR}=10$ s. To extract the $T_1$ values, the data were fitted to the equation:

$$M_z(t) = M_0 \sin \theta_{\text{flip}}(\cos \theta_{\text{flip}})^{\frac{t}{\text{TR}}} \exp(-t/T_1)$$

where $M_0$ is the original magnetisation prior to rf pulsing. This equation accounts for the loss of magnetisation due to rf excitation and $T_1$ relaxation.

![Diagram of pentaerythrityl tetraazide showing $T_1$ values for CH$_2$-linked, central, and terminal $^{15}$N nuclei.]

$T_1=64 \pm 2$ s

$T_1=147 \pm 4$ s

$T_1=122 \pm 4$ s

Fig. S6 Decay of the hyperpolarised $^{15}$N NMR signals from the CH$_2$-linked, central, and terminal $^{15}$N (natural abundance) nuclei of pentaerythrityl tetraazide (31 mM in methanol). These measurements were taken at 9.4 T and 298 K. The decay of the hyperpolarised signal was monitored by applying a 5-degree detection pulse every 10 s.
VII. UV/Vis spectroscopy of filtered and unfiltered DPPH solutions

Similar to the fast dissolution DNP of BDPA-doped samples, the hydrophobic DPPH free radical can be easily removed from aqueous dissolution liquids by a simple mechanical filtration process. Fig. S7 shows the UV/Vis spectra of varying DPPH concentration in methanol solution and the filtered aqueous dissolution liquid showing the absence of DPPH after filtration using a 0.2-micron syringe filter. The preparation of radical-free hyperpolarised solution is an important attribute for future in vivo magnetic resonance spectroscopy or imaging experiments.

![UV/Vis spectra of varying DPPH concentration in methanol solution and the filtered aqueous dissolution liquid.](image)

**Fig. S7** UV/Vis spectra of varying DPPH concentration in methanol solution and the filtered aqueous dissolution liquid.

VIII. References


