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Proton hyperpolarisation preserved in long-lived states

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The polarisation of abundant protons, rather than dilute nuclei with low gyromagnetic ratios, can be enhanced in less than 10 min using dissolution DNP and converted into a long-lived state delocalised over an ensemble of three coupled protons. This process is more straightforward than the hyperpolarisation of heteronuclei followed by magnetisation transfer to protons.

Recent advances have permitted the application of Dynamic Nuclear Polarisation techniques to enhance signals in high-resolution nuclear magnetic resonance (NMR). Dynamic Nuclear Polarisation (DNP) combined with the dissolution process affords the detection of dilute endogenous substances in NMR and MRI. The dramatic increase of nuclear spin polarisation is brought about by microwave irradiation of ESR transitions of a paramagnetic species, usually a stable radical, mixed with the substance to be analysed in frozen pellets at low temperatures (1.2 K) and moderate magnetic fields, typically at 3.35 or 5 T (94 or 140 GHz electron frequencies) as recently demonstrated. The rapid dissolution of polarised frozen pellets makes it possible to maintain most of the polarisation, yielding enhancements at room temperature that are up to four orders of magnitude compared to thermal Boltzmann polarisation at the same temperature. The dissolution, transfer from the polariser to the high-resolution magnet, and settling of the sample typically require 5 s in our laboratory. This causes significant losses due to the decay of polarisation. These polarisation losses, essentially arising from dipolar relaxation, are larger when the magnetic field is low during the voyage from one magnet to the other. It is possible to use custom-designed magnets with two distinct homogeneous regions to alleviate this problem, but this solution is not widely accessible. Applications of dissolution DNP have been mostly limited to low-γ nuclei with spins I = ½ because they have comparatively long spin-lattice relaxation times T1 at low fields. For instance, the long T1(15N) ≈ 189 s of partially-deuterated choline (CD3)315N+CH2CH2OHCl- in D2O can be used to preserve the enhanced 15N polarisation during the voyage, prior to transferring the magnetisation from 15N to 1H for detection.

Non-equilibrium populations of nuclear spins can be stored in the form of long-lived states (LLS) with lifetimes TLLS that can last much longer than the longitudinal 'spin-lattice' relaxation times T1. These states are largely immune to relaxation effects because of the spin-permutation symmetry of the system. An example are population differences between triplet and singlet states (SS) in isolated two-spin systems, which have long lifetimes because singlets are antisymmetrical with respect to spin permutation, which makes them immune to the symmetric dipolar interaction between the two involved spins. It was shown that long-lived spin states may be obtained in systems with more than two coupled spins, and that these states may be used to preserve enhanced polarisation. Besides their utility for the study of slow exchange and transport phenomena, with ratios as large as TLLS/T1 = 37 observed in pairs of diastereotopic protons in a partially deuterated saccharide, long-lived states were also shown to be useful for the study of molecular geometry.

An experiment combining both dissolution DNP and LLS has been designed where the magnetisation was transferred from a polarised carbonyl 13C (in natural abundance) to a pair of nearby H⁺ protons in the dipeptide Ala-Gly and stored in the form of a long-lived proton state with TLLS = 16 s in water at 298 K and 300 MHz (the ratio TLLS/T1 = 7 is expected to be further increased if the free radicals in the solution were quenched with sodium ascorbate). However, such experiments are time-consuming because they typically require about an hour to achieve an adequate 13C polarisation, P = (Pα - Pβ)/(Pα + Pβ) = 10%. A polarisation P(13C) = 10% corresponds to an enhancement of more than four orders of magnitude in comparison to the room-temperature Boltzmann distribution P(13C) = 0.6 × 10⁻³ at 300 MHz. However, if the transfer of magnetisation to protons starts from 13C in natural abundance (ca. 1%), the net enhancement of the protons compared to the room-temperature Boltzmann polarisation P(1H) = 2.5 × 10⁻⁵ at 300 MHz is only a factor of 40. The enhancement can of course be improved by 13C isotope labelling of the molecule under investigation, but this may be cumbersome and expensive.

In the present work, we show that enhanced long-lived proton states can be excited directly from hyperpolarised protons, without taking recourse to any dilute low-γ ‘transporter spins’ such as 13C or 15N. We start with a proton nuclear spin polarisation enhanced to about P(1H) = 40%, which can be achieved in less than 10 min by DNP at 1.2 K and 3.35 T. This does not require any isotope labelling. Reducing the transfer time in the dissolution DNP procedure to about 5 s and scavenging the TEMPO radicals during dissolution²⁴ make
it possible to limit $^1$H polarisation losses during the voyage. This leads to a better enhancement factor for direct proton DNP-LLS experiments. Not only does this allow one to achieve better sensitivity, but the $^1$H chemical shifts are also more sensitive than $^{15}$N or $^{13}$C shifts to biochemical modifications such as acetylation and phosphorylation.\(^9\)

So far, most applications of dissolution DNP have been limited to low-$\gamma$ ‘transporter spins’ such as $^{13}$C, $^{15}$N, etc., although it is possible to polarise water by the Overhauser effect at room temperature,\(^25\) yielding enhancement factors of about 10. We have observed enhancement factors of at least 1000 for hyperpolarised proton magnetisation, compared to the Boltzmann proton polarisation at 300 MHz and 298 K in small molecules such as acrylic acid.

A challenging system with three scalar-coupled protons was used to illustrate the potential of the new method, although these experiments are more straightforward in systems comprising only two $J$-coupled spins. In acrylic acid (CH$_2$H$^R$=CH$^COOH$) dissolved in D$_2$O (Fig. 1), long-lived states delocalised on the three protons H$^I$, H$^R$, and H$^S$ can have a lifetime $T_{\text{LLS}} = 52.5$ s with a ratio $T_{\text{LLS}}/T_1(H^S) = 2.3$.\(^17\) The purpose of the present experiment is to use these lifetimes in order to preserve hyperpolarised magnetisation. Frozen beads of acrylic acid in CD$_3$OD/D$_2$O were mixed with 50 mM TEMPOL and polarised in a home-built cryostat at 1.2 K and 3.35 T (94 GHz electron frequency) and then rapidly dissolved with hot water vapour, prior to the transfer to the NMR spectrometer. The spin-lattice relaxation times of protons H$^S$, under the present experimental conditions in the high-resolution magnet, $T_1(H^S) = 12$ s, are much longer than those of the other two protons in acrylic acid, $T_1(H^{IR}) = 5$ s, by virtue of the internuclear distances between the three protons. Therefore, protons H$^S$ also suffer the least during the voyage between the polarising and the high-resolution magnets and retain maximum polarisation, featuring an enhancement factor $a(H^S) = 1000$ (Fig. 1) compared to thermal equilibrium polarisation. Protons H$^R$ and H$^I$ lose a large fraction of their polarisation during the transfer.

Enhanced H$^S$ magnetisation is stored by conversion to a long-lived state (LLS) by the pulse sequence shown in Fig. 2. This sequence consists of two parts: (i) a preparation sequence, which converts the enhanced H$^S$ magnetisation into LLS and (ii) sustaining of the operator $Q^{\text{ISR}}$ (vide infra), prior to decoding and back-conversion to detectable magnetisation. The delays in the pulse sequence have been optimized numerically to achieve the largest possible coefficient of the delocalised long-lived state described by a linear combination operator product\(^17\) of the form:

$$Q^{ISR} = \frac{1}{\sqrt{3}}(\lambda_{IS} \cdot \hat{S} + \lambda_{IR} \cdot \hat{R} + \lambda_{SR} \cdot \hat{S} \cdot \hat{R})$$  \hspace{1cm} (1)

For acrylic acid, it can be shown that $\lambda_{IS} = -0.23$, $\lambda_{IR} = 0.93$, and $\lambda_{SR} = 0.28$. The delocalised long-lived state is sustained during a mixing time $\tau_m$ using a suitable radio-frequency (rf) scheme. The resultant proton signal intensities from each experiment are normalized by dividing the signal intensity by that of enhanced H$^S$, measured in a separate experiment with a 10$^\circ$ pulse, so as to account for variations in DNP enhancements in various experiments.

Nearly mono-exponential decays were obtained with $T_{\text{LLS}} = 51 \pm 4$ s (Fig. 3) when the sums of the normalized intensities were fitted. From experiments carried out under similar conditions without DNP, $T_{\text{LLS}} = 42 \pm 2$ s was obtained. It is noteworthy that similar enhanced long-lived states can be excited in common amino acids like serine, cysteine, aspartate, etc., where states with long lifetimes are described by linear combinations of operator products. In the case of serine we have observed an enhancement $\varepsilon = 350$ for isolated protons H$^S$.

We have demonstrated detection of hyperpolarised protons using dissolution DNP with significant enhancement factors. This method has several advantages over the hyperpolarisation of low-gamma nuclei: considerably improved detection sensitivity, due to the high gyromagnetic ratio and the 100%...
natural abundance of the detected nuclei, the sensitivity of their Larmor frequency to chemical changes (for studies of metabolic transformations), and the short polarising time. The use of enhanced long-lived proton magnetisation states circumvents the need for isotope labelling in order to preserve the magnetic spin state during metabolic transformations, and the short polarising time. This method is applicable to a wide range of systems of J-coupled protons and promising for the study of slow biochemical reactions.

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Notes and references


