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Puneet Ahuja,[a] Riddhiman Sarkar,†[a] Sami Jannin,[a] Paul R. Vasos*[ab] and Geoffrey Bodenhausen[ac]

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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. The 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).[1,2] Dynamic Nuclear Polarisation (DNP) combined with the dissolution process[1] affords the detection of dilute endogenous substances in NMR and MRI.[3,4] 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.[5–7] 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.[1] The dissolution, transfer from the polariser to the high-resolution magnet, and settling of the sample typically require 5 s in our laboratory.[4,5] 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.[8,9] Applications of *dissolution* DNP have been mostly limited to low-γ nuclei with spins $I = \frac{1}{2}$ because they have comparatively long spin-lattice relaxation times T_1 at low fields. For instance, the long $T_1(^{15}N) \approx 189$ s of partially-deuterated choline $(CD_3)_3{}^{15}N^+CH_2CH_2OH{\cdot}Cl^-$ in D_2O can be used to preserve the enhanced ^{15}N polarisation during the voyage, prior to transferring the magnetisation from ^{15}N to ^1H for detection.[6]

Non-equilibrium populations of nuclear spins can be stored in the form of long-lived states (LLS) with lifetimes T_{LLS} that can last much longer than the longitudinal 'spin-lattice' relaxation times T_1.[10–12] 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.[13–17] Besides their utility for the study of slow exchange and transport phenomena,[12,18–21] with ratios as large as $T_{LLS}/T_1 = 37$ 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.[22,23]

An experiment combining both *dissolution* DNP and LLS has been designed where the magnetisation was transferred from a polarised carbonyl ^{13}C (in natural abundance) to a pair of nearby H^α protons in the dipeptide Ala-Gly and stored in the form of a long-lived proton state with $T_{LLS} = 16$ s in water at 298 K and 300 MHz (the ratio $T_{LLS}/T_1 = 7$ is expected to be further increased if the free radicals in the solution were quenched with sodium ascorbate).[24] However, such experiments are time-consuming because they typically require about an hour to achieve an adequate ^{13}C polarisation, $P = (P_\alpha - P_\beta)/(P_\alpha + P_\beta) = 10\%$. A polarisation $P(^{13}C) = 10\%$ corresponds to an enhancement of more than four orders of magnitude in comparison to the room-temperature Boltzmann distribution $P(^{13}C) = 0.6 \times 10^{-5}$ at 300 MHz. However, if the transfer of magnetisation to protons starts from ^{13}C in natural abundance (*ca.* 1%), the net enhancement of the protons compared to the room-temperature Boltzmann polarisation $P(^1H) = 2.5 \times 10^{-5}$ at 300 MHz is only a factor of 40. The enhancement can of course be improved by ^{13}C 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 ^{13}C or ^{15}N. 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 TEMPOL radicals during dissolution[24] make

[a] *Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, EPFL, Batochime, 1015 Lausanne, Switzerland. E-mail: paul.vasos@epfl.ch; Fax: +41216939435; Tel: +412169 9435*
[b] *Université Paris Descartes, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601 CNRS, 75006 Paris, France. E-mail: paul.vasos@parisdescartes.fr; Tel: +33.1.42.86.21.80*
[c] *Département de Chimie, Associé au CNRS, UMR 7203, Ecole Normale Supérieure, 24 Rue Lhomond, 75231, Paris Cedex 05, France*
† *Present address*: School of Chemistry, University of Southampton, Southampton, UK SO17 1BJ.

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.[6]

So far, most applications of *dissolution* DNP have been limited to low-γ '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 ($CH^{I}H^{R}{=}CH^{S}COOH$) dissolved in D_2O (Fig. 1), long-lived states delocalised on the three protons H^{I}, H^{R}, and H^{S} can have a lifetime T_{LLS} = 52.5 s with a ratio $T_{LLS}/T_1(H^{S})$ = 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_3OD/D_2O 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, $T_1(H^{I,R})$ = 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 $\varepsilon(H^{S})$ = 1000 (Fig. 1) compared to thermal equilibrium polarisation. Protons H^{R} and H^{I} lose a large fraction of their polarisation during the transfer.

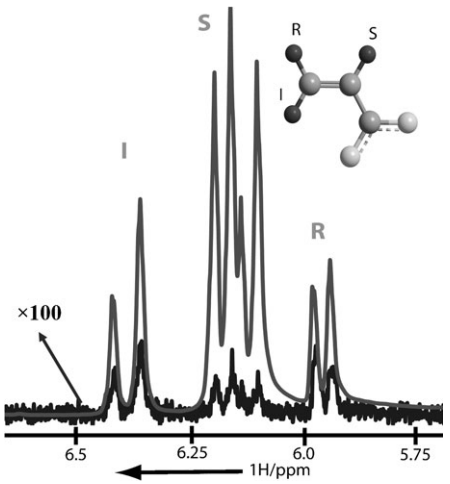

Fig. 1 Enhanced spectrum (grey) of hyperpolarised acrylic acid obtained 5 s after dissolution of a dynamically polarised sample. The free induction decay was excited with a 10° pulse. The conventional spectrum (dark line, ×100) was obtained at thermal equilibrium under similar conditions. An enhancement factor ε = 1000 is observed for H^{S} in the central region. The sample contained 1 M acrylic acid mixed with 50 mM 2,2,6,6-tetramethylpiperidine-1,4-diol (TEMPOL) in 50/50 v/v CD_3OD/D_2O. The frozen beads were placed in a home-built polariser[4] and irradiated at 93.85 GHz with 30 mW microwave power for 10 min at 1.2 K and 3.35 T. The sample was rapidly dissolved in 5 ml D_2O (preheated to 120 °C at 1 MPa) to a final choline concentration of 8 mM. The hyperpolarised solution was transferred to a 5 mm tube maintained at a temperature of 25 °C in an inverse broadband probe in a high-resolution 300 MHz (B_0 = 7.05 T) magnet.

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_l^{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:

$$Q_l^{ISR} = \frac{1}{\sqrt{3}}(\lambda_{IS}\vec{I}\cdot\vec{S} + \lambda_{IR}\vec{I}\cdot\vec{R} + \lambda_{SR}\vec{S}\cdot\vec{R}) \qquad (1)$$

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 τ_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° pulse, so as to account for variations in DNP enhancements in various experiments.

Nearly mono-exponential decays were obtained with T_{LLS} = 51 ± 4 s (Fig. 3) when the sums of the normalized intensities were fitted. From experiments carried out under similar conditions without DNP, T_{LLS} = 42 ± 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 ε = 350 for isolated protons H^{α}.

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%

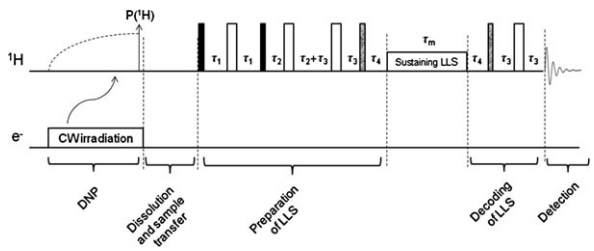

Fig. 2 The pulse sequence used for creating a hyperpolarised LLS delocalised on acrylic acid protons, starting with $P(H^{S})$ = 40%. The enhanced magnetisation is followed by the preparation of a long-lived state described by eqn (1) and conversion back into detectable magnetisation.

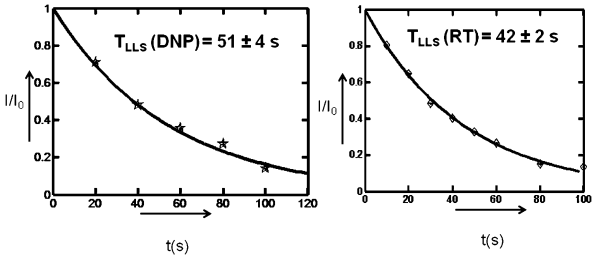

Fig. 3 (Left) Fits of exponential decays of DNP-enhanced LLS in hyperpolarised acrylic acid. (Right) LLS experiments carried out without DNP at room temperature (RT), under similar conditions. For each hyperpolarised sample an experiment with a 10° pulse for normalization was carried out. A continuous-wave (CW) *rf* field with an amplitude $\nu_1 = 2.5$ kHz and a carrier frequency half-way between the chemical shifts of protons H^I and H^R was used to sustain the LLS.

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 magnetisation on nuclei with low gyromagnetic ratios. 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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