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Hyperpolarized ^{1}H NMR Employing Low γ Nucleus for Spin Polarization Storage

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The PASADENA (parahydrogen and synthesis allow dramatically enhanced nuclear alignment)[1,2] and DNP (Dynamic Nuclear Polarization)[3] methods efficiently hyperpolarize biologically relevant nuclei such as ^{1}H, ^{31}P, ^{13}C, ^{15}N achieving signal enhancement by a factor of ~100 000 on currently utilized MRI scanners. Recently, many groups have demonstrated the utility of hyperpolarized MR in biological systems using hyperpolarized ^{13}C biomarkers with a relatively long spin lattice relaxation time T_1 on the order of tens of seconds.[4−7] Moreover, hyperpolarized ^{15}N for biomedical MR has been proposed due to even longer spin lattice relaxations times.[8] An additional increase of up to tens of minutes in the lifetime of hyperpolarized agent *in vivo* could be achieved by using the singlet states of low gamma (γ) nuclei.[9] However, as NMR receptivity scales as γ^3 for spin $^1/_2$ nuclei, direct NMR detection of low γ nuclei results in a lower signal-to-noise ratio compared to proton detection. While protons are better nuclei for detection, short spin lattice relaxation times prevent direct ^{1}H hyperpolarized MR in biomedical applications.

Here, we demonstrate the utility of ^{13}C for spin storage of hyperpolarization followed by ^{1}H detection using INEPT,[10] which theoretically can provide up to an $\sim(\gamma_{1H}/\gamma_X)^2$ gain in sensitivity in hyperpolarized biomedical MR. Specifically, we hyperpolarized the ^{13}C site of two well studied molecules, 1-^{13}C-succinate-d_2[5,11] and 2,2,3,3-tetrafluoropropyl 1-^{13}C-propionate-d_3 (TFPP), by PASADENA (Figure 1). Both molecules are accessible from unsaturated precursors containing a double bond by molecular cis addition of parahydrogen. Hyperpolarized succinate[5,11] can be potentially exploited as a metabolic biomarker of cancer, while hyperpolarized TFPP has been shown to be a specific binder to lipids with a unique chemical shift signature in the lipid bound state[12] potentially useful for plaque imaging.[13]

Parahydrogen addition and transfer of spin order to ^{13}C has been described previously.[5,14] A home-built PASADENA polarizer was employed to hydrogenate 2,2,3,3-tetrafluoropropyl 1-^{13}C-acrylate-d_3 (TFPA) to yield hyperpolarized TFPP and 1-^{13}C-fumarate-d_2 (CIL, Andover, MA) to yield hyperpolarized 1-^{13}C-succinate-d_2 in deuterated solvent. The spin order transfer was performed using an untuned saddle coil at 1.76 mT utilizing the heteronuclear spin order transfer pulse sequence described by Goldman and Johannesson[15] and was tailored to the hetero- and homonuclear J coupling of propionate[14,16] for TFPP and succinate at pH 11 (Figure 2).[5]

In vitro 1-^{13}C succinate spin lattice relaxation time T_1 is 105 ± 1 s in D_2O at pH 11, and *in vivo* T_1 is in excess of 43 s at 4.7 T, which is significantly longer than the previously published values at pH 3.[5] *In vitro* 1-^{13}C TFPP $T_1 = 67 \pm 1$ s in deuterated medium, and *in vivo* T_1 is in excess of 16 s at 4.7 T.[13] Such long spin lattice relaxation times provide an efficient storage of spin polarization in long-lived low γ nuclear spin states, which is exemplified here by ^{13}C TFPP and succinate. The principal motivation for development of long-lived nuclear spin states is their utility to monitor biochemical pools *in vivo* such as stable isotope enrichment of metabolic events or receptor binding. NMR signal detection utilizing polarization transfer from long-lived low γ nuclear spin states to J-coupled protons provides a potential to further increase the MR signal in such studies (Figure 2) utilizing the strategy of two sequential polarization transfers.[17]

In one experiment, 2.4 mL of 6.2 mM 1-^{13}C-succinate-d_2 were hyperpolarized at the ^{13}C site to 10.7%. Hyperpolarization was then kept on ^{13}C for 70 s. During this time, the polarized sample was

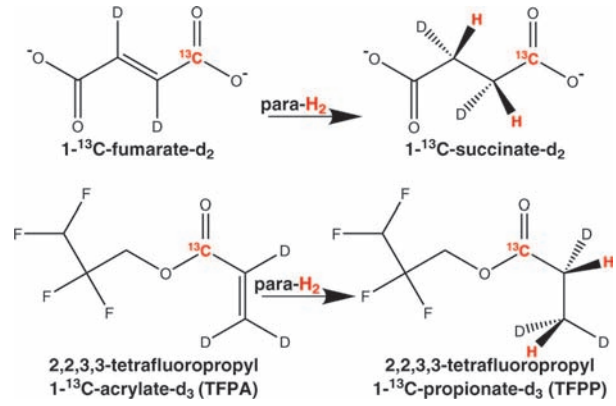

Figure 1. Cis molecular addition of parahydrogen to 1-^{13}C-fumarate-d_2 to produce 1-^{13}C-succinate-d_2 and cis molecular addition of parahydrogen to TFPA to produce TFPP. The catalytic reaction was carried out at 60 °C in D_2O with reactant concentrations of 3−6 mM. TFPP aqueous solution used 10% v/v acetone-d_6 necessary to dissolve hyperpolarized product.

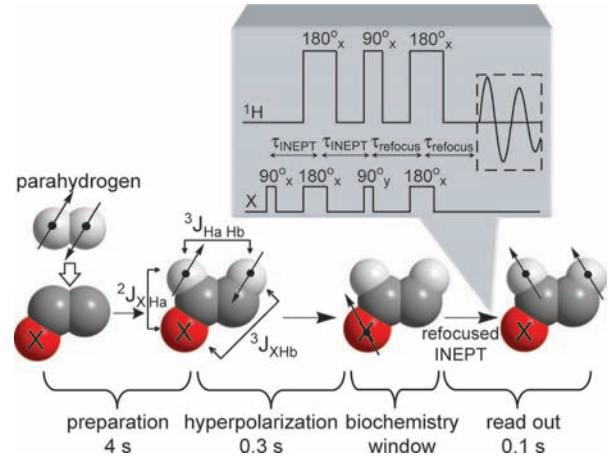

Figure 2. The experimental diagram of molecular cis addition of parahydrogen followed by hyperpolarization of X nucleus exemplified by ^{13}C, polarization storage on X nucleus (potentially allowing monitoring of biochemical events on the time scale of minutes) followed by polarization transfer back to more sensitive protons for NMR detection.

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transferred from a low magnetic field polarizer operating at 1.76 mT to a 4.7 T animal MR scanner. The ^{13}C polarization decayed from 10.7% to 5.5% corresponding to the final ^{13}C signal enhancement by a factor of 13 500. Then the refocused INEPT pulse sequence[10] with $\tau_{INEPT} = 34$ ms and $\tau_{refocus} = 32$ ms (Figure 2) was used to transfer polarization from ^{13}C to protons within 1-^{13}C-succinate-d_2 (Figure 3A and 3B). We found that the two protons were successfully hyperpolarized corresponding to 41% polarization transfer efficiency and 1350-fold ^1H NMR signal enhancement per two methylene protons. In a separate experiment, 2.4 mL of 2.9 mM TFPP were polarized to 14%, and the hyperpolarization was stored on the 1-^{13}C site for 24 s, during which the polarization decayed to 9.5% corresponding to a final signal enhancement of 23 300-fold at this site (Figure 3E). The delays of the refocused INEPT were $\tau_{INEPT} = 20$ ms and $\tau_{refocus} = 16$ ms. The combined intensity of the three NMR lines corresponding to four hydrogen atoms (Figure 3F) was enhanced by a factor of 2930, corresponding to the 50% polarization transfer efficiency by the refocused INEPT sequence.

To quantify the degree of hyperpolarization, we used the reference of a single scan spectrum of thermally polarized 100% natural abundance ethanol (Figure 3A) and 3 M sodium 1-^{13}C-acetate (Figure 3B) at 4.7 T using the formula:

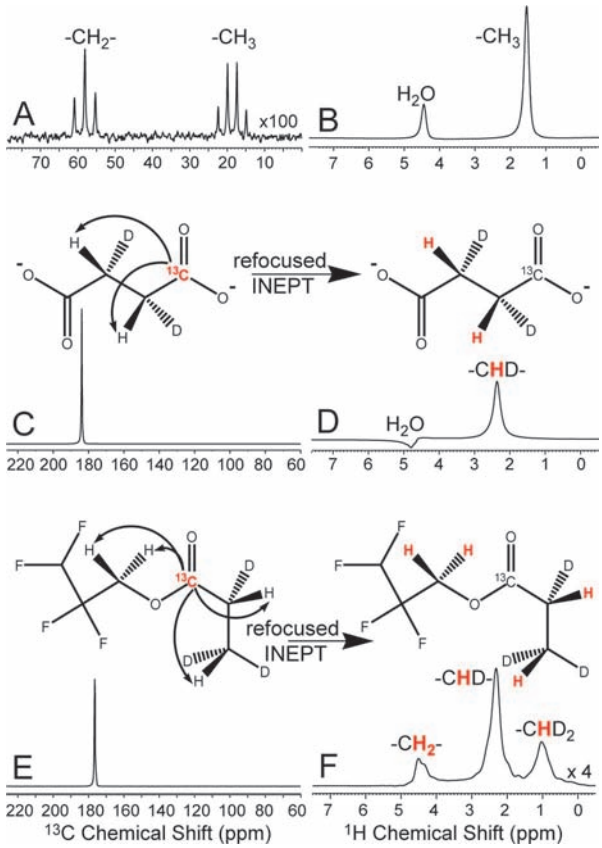

Figure 3. (A) ^{13}C reference spectrum of 2.8 mL of 17 M ethanol with 188 mM ^{13}C concentration per site. (B) ^1H NMR spectrum of 2.8 mL of 3 M sodium ^{13}C-acetate in D_2O. (C) ^{13}C NMR spectrum of hyperpolarized 6.2 mM 1-^{13}C-succinate-$d_{2,3}$, ^{13}C polarization of 5.5% after being stored for 70 s, $T_1 = 105$ s, the spectrum is acquired using a 12° excitation pulse. (D) ^1H NMR spectrum of hyperpolarized 6.2 mM 1-^{13}C-succinate-$d_{2,3}$ where net ^1H signal enhancement is 1350-fold with 41% spin polarization transfer efficiency. (E) ^{13}C NMR spectrum of hyperpolarized 2.9 mM TFPP. ^{13}C polarization is 9.5% after being stored for 24 s, $T_1 = 67$ s. The spectrum is acquired using a 12° excitation pulse. (F) ^1H NMR spectrum of hyperpolarized 2.9 mM TFPP where net ^1H signal enhancement is 2930-fold with 51% efficiency.

$$\%P_X = \frac{\chi_{ref}}{\chi_{expt}} \cdot \frac{S_{expt}}{S_{ref}} \cdot \frac{P_X^o}{\sin\theta} \cdot 100\%$$

where P_X^o is the nuclear polarization at equilibrium at 298 K and 4.7 T, according to the Boltzmann distribution, θ is the angle of the detection pulse, χ_{ref} and χ_{expt} are the molar concentrations of sites in the reference and the experimental molecule, respectively, and S_{ref} and S_{expt} are the signal from the reference and experimental molecular sites, respectively. Under the experimental conditions, $P_{^{13}C}^o$ is 246 600^{-1} and $P_{^1H}^o$ is 62 000^{-1}. The achieved $\%P_{1H}$ was 2.2% for 1-^{13}C-succinate-d_2 and 4.8% for TFPP. The efficiency of the polarization transfer from ^{13}C to ^1H reported here, 41% for 1-^{13}C-succinate-d_2 and 50% for TFPP, is a ratio between the ^1H polarization detected after the transfer and ^{13}C polarization as measured by a 12° excitation pulse before the INEPT transfer. While the efficiency of the polarization transfer was 50% or below, hyperpolarized protons are inherently 15.8-fold more sensitive compared to hyperpolarized ^{13}C. As a result, proton detection of hyperpolarized 1-^{13}C-succinate-d_2 and TFPP increased the overall sensitivity by a factor of 6.5 and 7.9, respectively.

The method demonstrated herein can potentially be applied to these and other hyperpolarized ^{13}C metabolic contrast agents *in vivo* including hyperpolarized pyruvate,[18] lactate, bicarbonate,[6] alanine, glutamine,[7] and choline.[8] More importantly, using this approach, hyperpolarized ^{15}N MR would become an attractive biomedical tool due to the much longer spin lattice relaxation time owing to low γ, but now with the added advantage of more sensitive detection using proton NMR ($\gamma^2_{15N} \approx \gamma^2_{1H}/100$). Furthermore, proton imaging, localized spectroscopy, and chemical shift imaging (CSI) will allow improved spatial resolution by γ_{1H}/γ_X in each dimension at a given gradient strength.

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