Sampling Hyperpolarized Molecules Utilizing a 1 Tesla Permanent Magnetic Field

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Hyperpolarized magnetic resonance spectroscopy (HP MRS) using dynamic nuclear polarization (DNP) is a technique that has greatly enhanced the sensitivity of detecting 13C nuclei. However, the HP MRS polarization decays in the liquid state according to the spin-lattice relaxation time (T1) of the nucleus. Sampling of the signal also destroys polarization, resulting in a limited temporal ability to observe biologically interesting reactions. In this study, we demonstrate that sampling hyperpolarized signals using a permanent magnet at 1 Tesla (1T) is a simple and cost-effective method to increase T1s without sacrificing signal-to-noise. Biologically-relevant information may be obtained with a permanent magnet using enzyme solutions and in whole cells. Of significance, our findings indicate that changes in pyruvate metabolism can also be quantified in a xenograft model at this field strength.

Nuclear magnetic resonance (NMR) spectroscopy of biologically relevant nuclei, aside from protons, has long been limited by the lack of sensitivity. The development of hyperpolarization (HP) techniques has led to a dramatic increase in the signal-to-noise ratio in solution state. HP can be achieved by a number of methods including optical pumping1, para-hydrogen-induced polarization (PHIP)2,3 as well as dissolution dynamic nuclear polarization (DNP)4. Of these, DNP has received the most attention with a clinical trial recently completed5.

Regardless of how HP is achieved, the polarization decays in the liquid state according to the spin-lattice relaxation time (T1) of the nucleus. Sampling of the signal also uses polarization, resulting in a limited temporal ability to observe biologically interesting information if small flip angles are not employed. There have been several methods attempted to preserve nuclear polarization after dissolution. One approach involves converting polarization to a nuclear singlet-state order that can be stored and accessed. This can be accomplished through field cycling, continuous irradiation or chemical modification but none of these methods are compatible with biologically relevant imaging systems6. A more recent experiment utilized a 13C-labeled molecule with coupling constants that exceed the chemical shift difference demonstrated a modest increase in T17. Currently, the singlet-state can only be accessed with a limited class of chemical structures and a more practical method of increasing the lifetime of polarization is by substituting exchangeable protons with deuterium (2H). This has been demonstrated with a number of different metabolites8–10, with reasonable increases in T1, although this method might be prohibitive due to the high costs involved in synthesizing deuterated substrates, limiting for screening large libraries of non-labeled compounds, as well as the potential for an isotope effect slowing biochemical kinetics11.

Many of the nuclear spins chosen for 13C isotopic enrichment and exploration tend to be carbonyl carbons since they are devoid of directly attached relaxation centers (protons) and are present in many biological systems15. Unlike in quaternary carbons, where T1 relaxation is dominated by dipole-dipole relaxation, carbonyl carbons additionally suffer from chemical shift anisotropy (CSA) or wobble induced relaxation. The T1 relaxation time under CSA is inversely proportional to the square of the external magnetic field strength (B02). Therefore, in addition to the methods described to prolong the T1, sampling of the HP signal in a lower external magnetic field would result in prolonging the T1s of the molecules. To our knowledge, there have been two previous studies investigating the effects of external field strengths on T1. Chattergoon and colleagues investigated the field dependence of T1 for HP [1-13C]pyruvate using field-cycled relaxometry. From field strengths between 0.237 mT

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to 0.705 T, the authors observed a steady increase in $T_1$. Meanwhile, Mieville and colleagues investigated the $R_1$ (equivalent to the reciprocal of $T_1$), which reached a minimum at fields close to 1T. Here, we hypothesize that sampling the HP signal using a permanent magnet at 1T will be a simple and cost effective way to significantly increase $T_1$ values, regardless of the method of HP. The majority of MRI and NMR spectroscopy have utilized large, cryogenically cooled superconducting magnets to establish the static $B_0$ field. To the best of our knowledge, the use of a permanent, 1T spectrometer has yet to be demonstrated with hyperpolarized MR.

**Results**

We demonstrate that the shim achieved with a permanent magnet is satisfactory (Supp. Fig. S1). Additionally, with a pre-saturation pulse, the NMR signal derived from water can be suppressed to allow the visualization of biologically relevant compounds in such as choline (Supp. Fig. S2). Figure 1 demonstrates the clear distinction and measurement of the spin-spin coupling between the $^{13}$C-enriched carbon (99% $^{13}$C) of $[1-^{13}$C] pyruvate and the adjacent natural abundance C2 and C3 carbons (1.1% $^{13}$C). Excellent signal-to-noise ratios (SNR) enable observation of these scalar couplings within the first scan of the experiment, allowing rapid determination of the chemical environment surrounding the labeled atom. Interestingly, the coupling between carbons and protons is also visible at this field strength by acquiring data without $^1$H decoupling. Carbons attached to adjacent protons displayed a distinctive 13.4 Hz doublet while a 62.1 Hz doublet was observed due to C1-C3 and C1-C2 coupling (Fig. 1B).

To demonstrate that sampling at 1T preserves the $T_1$ of a variety of molecules, we proceeded to polarize and dissolve a range of molecules enriched at different functional groups. Table 1 summarizes the $T_1$ of HP compounds that we have measured at 1T compared to literature values. There was an appreciable lengthening of the $T_1$ times across a wide range of carbonyl containing chemical structures, including keto-acids and amino acids. As expected non-carbonyl carbons, or carbons with minimal CSA, did not have significantly increased $T_1$s.

To demonstrate the feasibility of the system to quantify biologically-relevant reactions, we measured the kinetics of lactate dehydrogenase (LDH) that catalyzes the conversion of pyruvate to lactate. The use of a low-field permanent magnet enabled the close positioning of the spectrometer to the hyperpolarizer, enabling multiple measurements from a single dissolution. Figure 2A depicts a representative experiment where 4 mM of hyperpolarized pyruvate was injected into a 5 mm NMR tube containing 0.4 U of LDH. The data were fit to a model of interconversion from pyruvate to lactate to produce a flux rate, $k_{PL}$ (Fig. 2B). By repeating the experiment using different concentrations of pyruvate, we were able to quantify the kinetics of LDH, with a $V_{max}$ value of 0.024 mM/sec and

![Figure 1](image-url)

**Figure 1.** (A) Schematic of the spontaneous conversion of a solution containing 1.6 μmol of hyperpolarized $[1-^{13}$C] pyruvate (in the active region of the coil) to $[1-^{13}$C] pyruvate hydrate in a buffered solution at pH 7.4 at RT. This conversion can be observed in the first scan acquired using a 1T permanent magnet, with the scalar coupling (J coupling) of the different carbon functional groups easily observed. Coupling, between C1 and C2 of pyruvate results in a doublet separated by 62.1 Hz while coupling between C1 and C3 is separated by 13.4 Hz (B). Proton-carbon couplings can also be observed at this field strength (C), as evidenced by a quartet separated by 1.5 Hz.
a $K_m$ value of 0.82 mM (Fig. 2C). The $K_m$ value obtained approximates literature values$^{23}$, suggesting that hyperpolarized MRS might be an alternative method to accurately quantify enzyme kinetics where no other assays are available. The majority of HP experiments in perfused bioreactors have been performed on high field NMR systems$^{22,24}$ (e.g. 11.7–14.1T). These experiments require a constant flow of growth media, gaseous exchange and temperature control. Integration of these requirements onto existing NMR systems can be cumbersome and risks damaging expensive, superconducting systems. In contrast, a permanent 1T magnet has a small footprint, allowing perfusion and gaseous exchange platforms to be built around it. In an experiment of alginate-encapsulated PC3 prostate cancer cells, an injection of hyperpolarized [1-13C] pyruvate resulted in the production of [1-13C] lactate but with a quarter of the number of cells used in previous publications$^{25}$ (Supp. Fig. S3A). We were also able to detect peaks of total choline (tCho) and lactate, using proton spectroscopy after the application of a water saturation pulse (Supp. Fig. S3B).

We went on to demonstrate the feasibility of acquiring spectroscopic imaging data from hyperpolarized substrates in live animals. A subcutaneous GIST-T1 sarcoma was subcutaneously xenografted on a mouse and injected with 0.05 mg/kg of [1-13C] pyruvate. While the resultant [1-13C] lactate was visible in regions of the muscle and kidney, this peak was evidently larger in tumor regions (Fig. 3A). This spectroscopic data can also be used to create false-color images using the same 1T permanent magnet, allowing localization of regions of high lactate production when superimposed on regular anatomical images (Fig. 3B). To demonstrate that we were able to detect cancer treatment response, tumors were treated with rapamycin, a potent inhibitor of the mammalian

### Table 1. Apparent spin-lattice relaxation times ($T_1$) for various hyperpolarized molecules at 1 Tesla compared to previously published values. All metabolites were dissolved in a buffered solution at pH = 7.4 and measurements performed at 25 °C.

| Compound         | HP $T_1$ at 1T | Literature $T_1$ |
|------------------|----------------|-----------------|
| [1-13C] pyruvate | 71.3 ± 1.8s    | 40.0 s (9.4T)$^{16}$ |
| [1-13C] glutamate| 66.5 ± 6.4s    | 33.9 s (9.4T)$^{17}$ |
| [1-13C] oxalate  | 66.7 ± 7.0s    | n/a             |
| [1-13C] lactate  | 42.9 ± 4.2s    | 33s (14.1T)$^{18}$ |
| [13C-15N] urea   | 50.7 ± 1.8s    | 44s (3T)$^{19}$ |
| [1-13C] methionine| 47.6 ± 2.8s   | 17s (9.4T)$^{20}$ |
| [1-13C] dehydroascorbate | 75.1 ± 3.1s | 57s (3T)$^{21}$ |

![Figure 2](image-url)
target of rapamycin (mTOR). Rapamycin treatment has already been shown to decrease glycolytic flux to lactate and can be detected using radioactive PET imaging. In Fig. 3D, we demonstrate that by using our small animal imaging setup, a significant decrease of 38% (p-value < 0.01) of hyperpolarized lactate was observed 24 hrs post-rapamycin treatment.

Discussion

HP MRI has been informative in many fields including tumor metabolism, cardiac biology as well as inflammation. There has been much excitement over the potential of this imaging modality and a simple, cost-effective method to sample hyperpolarized molecules will ensure that this technology can be adopted more extensively. In a literature search on Pubmed with the terms 'hyperpolarized' and 'dynamic nuclear polarization', we assessed 185 publications dating back to 2002 and uncovered no instances of HP experiments performed on permanent magnets with the lowest field strength recorded was a 2.4 T super-conducting, small animal imaging system. Therefore, we believe that this study represents the first systematic demonstration that HP MRI can be reliably performed using permanent magnets at low field. While the drift of a permanent magnet can be significant, the availability of field locks ensures this potential problem will be mitigated. Additionally, the lifetime of the HP experiment is in the order of seconds to minutes, ensuring that intra-experimental drift will be negligible. Sampling at lower field strengths will result in significant homonuclear 13C couplings resulting in the observation of multiplets in the spectra. This might complicate quantification, but we also believe that this might be an advantage in elucidating molecules of unknown structure. We also observe intensity asymmetries in the doublet centered at 27 and 206 ppm. We attribute this asymmetry to differential polarization of the coupled 13C nuclei, consistent with observations made previously. While there has been much excitement for the translation of HP MRI to the clinic, the majority of hospital scanners operate at 1.5T. If HP MRI is to be widely tested in multi-center human trials, an understanding of the behavior of molecules at clinically-relevant field strengths would be essential. There are a number of other benefits from using a low-field system, including less susceptibility effects, ease of use as well as the ability to rapidly integrate MR with other imaging modalities such as PET. We believe that HP experiments in vivo will especially benefit from the lengthened T1s at 1T because the longitudinal relaxation time of HP molecules have been shown to be shorter in vivo as compared to in solution. Longer T1s will also enable the simultaneous measurement of a multi-enzyme cascade, where the fate of the polarized metabolite is known, from a single dissolution. The effect of T1 lengthening is by no means a unique property of hyperpolarized measurements at 1T, nor do they represent the maximum T1 values obtainable. However, the availability of benchtop spectrometers at this field strength and ease of use adds to attractiveness of sampling hyperpolarized molecules at 1T. Additionally, the majority of hyperpolarized metabolites are small molecules with correlation times that will benefit from sampling at lower field strengths. Furthermore, co-polarization studies can also be performed to measure kinetics of several different metabolites being acted upon by different enzymes. Previous studies polarizing multiple compounds simultaneously utilized a 3T magnet and we believe similar, quantifiable results should be obtainable using a 1T permanent magnet. In summary, we believe sampling hyperpolarized molecules at low-field has both practical benefits besides providing novel information.
imaging, beads were inserted into a custom-built 5 mm NMR-compatible bioreactor. HP [1-13C] pyruvic acid was used with NADH concentrations of 1 mM. The hyperpolarized pyruvate-to-lactate flux was quantified using a previously published model22.

The angle, PE = \frac{\pi}{2} - \frac{\pi}{2} \sin(2\alpha)

was extruded through a 23 G angiocatheter to beads of approximately 500 μm (St. Louis, MO). 13C metabolites were prepared for HP according to published reports: [1-13C] pyruvate 24, [13C,15N2] urea36, [1-13C] dehydroascorbate and [1-13C] ascorbate 21. All metabolites were HP using a prototype SpinLab (General Electric, Niskayuna, New York, USA) for approximately 90 min before dissolving with appropriate buffers as described in previous publications.

NMR and T1 determination. NMR studies were performed on a 1 Tesla Magritek Spectrometer (Magritek, San Diego, CA) using a 5 mm H13C coil. The dissolution process, transfer of solution and transit to the spectrometer was approximately 20 s and this was accounted for in all T1 calculations. All transfer was performed in the presence of a permanent magnet to avoid depolarization. For the acquisition of HP spectra in solution, a repetition time (TR) of 3 s was applied for a total of at least 64 scans. For T1 measurements, HP solutions were placed into a pre-warmed NMR tube and a series of spectra was collected with 3 s temporal resolution. These spectra were then fit to a mono-exponential decay function, correcting for flip angle, to determine the spin-lattice relaxation time as previously describedd6.

Cell culture, enzyme and bioreactor experiments. PC3 prostate cancer and GIST-T1 sarcoma cells were grown under standard conditions. For experiments with LDH-A pure enzyme, commercial rabbit LDH-A (Sigma-Aldrich, St. Louis, MO) was diluted in Tris-HCL buffer, pH 7.4. For kinetic experiments, 1 U enzyme was used with NADH concentrations of 1 mM. The hyperpolarized pyruvate-to-lactate flux was quantified using a previously published model22.

For bioreactor experiments, 92 hr before imaging, cells were trypsinized, pelleted and resuspended in a sodium alginate solution in HBSS at a concentration of 1 × 10⁶ cells/ml. After thorough mixing, the cell suspension was extruded through a 23 G angiocatheter to beads of approximately 500 μm in diameter. On the day of imaging, beads were inserted into a custom-built 5 mm NMR-compatible bioreactor. HP [1-13C] pyruvic acid was dissolved and injected into the perfusion system. Spectra were acquired using a 10° excitation pulse every 10 seconds. A section of the bead was fixed in 10% neutral buffered formalin for histology while the rest dissociated for trypan blue exclusion and cell counting.

For water suppression, a presaturation pulse at 18.3 Hz at an amplitude of − 25 dB and 2000 ms duration was applied at 1000 ms repetition time, 11 us pulse length, 20 us acquisition delay and a gradient amplitude of 7000.

In vivo experiments. The animal portion of this study was performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). 5 × 10⁶ GIST-T1 cells were trypsinized and resuspended in a 1:1 solution of complete media: Matrigel. A total of 6 animals were subcutaneously injected on the flank of NOD/SCID mice. Mice were imaged utilizing a hybrid 1T MR/PET system (Mediso, USA) equipped in the presence of a permanent magnet to avoid depolarization. For the acquisition of HP spectra in solution, a relaxation time as previously described8.

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Author Contributions
S.S.T. and V.D.G. conceived, designed, performed experiments, analyzed data and prepared manuscript. R.E., S.J., K.L.G., V.M., A.J.P., S.T., J.A.A. and H.N.A. performed experiments and analyzed data. K.R.K. conceived, designed, performed experiments, analyzed data and prepared manuscript. R.E., S.S.T. and V.D.G. conceived, designed, performed experiments, analyzed data and prepared manuscript. R.E., S.S.T. and V.D.G. conceived, designed, performed experiments, analyzed data and prepared manuscript.

Additional Information
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