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High-Resolution Hyperpolarized In Vivo Metabolic ^{13}C Spectroscopy at Low Magnetic Field (48.7 mT) Following Murine Tail-Vein Injection

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Abstract

High-resolution ^{13}C NMR spectroscopy of hyperpolarized succinate-1- ^{13}C is reported *in vitro* and *in vivo* using a clinical-scale, biplanar (80 cm-gap) 48.7 mT permanent magnet with a high homogeneity magnetic field. Non-localized ^{13}C NMR spectra were recorded at 0.52 MHz resonance frequency over the torso of a tumor-bearing mouse every 2 seconds. Hyperpolarized ^{13}C NMR signals with linewidths of ~3 Hz (corresponding to ~6 ppm) were recorded *in vitro* (2 mL in a syringe) and *in vivo* (over a mouse torso). Comparison of the full width at half maximum (FWHM) for ^{13}C NMR spectra acquired at 48.7 mT and at 4.7 T in a small-animal MRI scanner demonstrates a factor of ~12 improvement for the ^{13}C resonance linewidth attainable at 48.7 mT compared to that at 4.7 T *in vitro*. ^{13}C hyperpolarized succinate-1- ^{13}C resonance linewidths *in vivo* are at least one order of magnitude narrower at 48.7 mT compared to those observed in high-field (~3 T) studies employing HP contrast agents. The demonstrated high-resolution ^{13}C *in vivo* spectroscopy could be useful for high-sensitivity spectroscopic studies involving monitoring HP agent uptake or detecting metabolism using HP contrast agents with sufficiently large ^{13}C chemical shift differences.

Graphical Abstract

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web site.

Keywords

NMR; hyperpolarization; parahydrogen; ¹³C; *in vivo* spectroscopy

1. Introduction

The emergence of several hyperpolarization techniques enhancing the detected signal from nuclear spins by orders of magnitude [1–5] have led to a wealth of opportunities to pursue in the developing arena of high sensitivity magnetic resonance research. In non-hyperpolarized methods, the magnetic field strength of the detection magnet serves to polarize the nuclear spins (usually protons) with polarization levels of ~3.5 ppm/Tesla constraining the detection limit [6] to large ensembles of nuclear spins (conventionally bulk water in clinical MR applications). Hyperpolarization techniques, however, produce spin polarization independent of the readout magnet, and therefore, they provide freedom to select a magnetic field strength, and correspondingly a detection frequency, optimized to the nucleus observed [7, 8] and the dielectric properties of the sample [9–11]. Of particular interest is the quickly developing frontier of low-field magnetic resonance (NMR) and imaging (MRI) [12–14], due to its ability to strongly complement the study of hyperpolarized (HP) nuclei, and its potential for application in areas of study including heteronuclear spin-labeled metabolites [15, 16] or other contrast agents [17–22] at low concentrations.

Key advantages have given rise to the desirability of pursuing low-field NMR and MRI. First, detection at low static magnetic field B_0 is known to yield far less vulnerability to magnetic susceptibility-induced field gradient artifacts in imaging, including those surrounding metallic implants [23] or interfaces between materials such as airways and tissues as occur in the lungs, inner ear, and nasal passages, thus significantly reducing the advanced shimming hardware requirements [24]. Second, the specific absorption rate (SAR) of energy deposited in the sample/subject during application of radiofrequency (RF) pulses scales $\propto B_0^2$. Consequently, lowering the field strength confers the benefit of reducing SAR to potentially negligible levels [11], which not only alleviates patient safety concerns, but can also lead to design and implementation of faster pulse sequences by permitting use of RF pulses for echoes instead of gradient echoes [11]. Third, through utilizing long-lived spin states (LLSS) [25–36] or taking advantage of reduced contributions from chemical shift anisotropy particularly below 0.1 T [37], hyperpolarized state lifetimes can significantly extend for protons and/or heteronuclei at low magnetic fields. Fourth, experimental validation of the theoretical equations for low-field NMR and MRI [12, 13] sensitivity for

hyperpolarized states has favorably corroborated the weak frequency dependence of hyperpolarized state detection, where these theories indicate the potential for approaching or even exceeding the sensitivity of high-field NMR/MRI [12, 13]. Lastly, utilization of low-field magnets provides significantly less expensive purchasing and maintenance costs, which can pair favorably with lower-cost methods of producing hyperpolarized spin states such as Spin Exchange Optical Pumping (SEOP) primarily focused on ^3He and ^{129}Xe [1, 38–42] or the high-throughput parahydrogen-based methods [5, 14, 43] including parahydrogen-induced polarization (PHIP) [44–46] and signal amplification by reversible exchange (SABRE) [47–51].

In this communication, we demonstrate proof-of-principle high-resolution ^{13}C NMR spectroscopic detection of HP succinate-1- ^{13}C -2,3- d_2 (^{13}C -SUX) at $B_0 = 47.5$ mT *in vitro* as well as *in vivo*. Here, we take advantage of the significantly more homogeneous B_0 field generated by a clinical-scale 80 cm-gap biplanar permanent magnet with ~ 13 ppm homogeneity over 40 cm diameter spherical volume (DSV) compared to a previously employed 8.9 cm-bore magnet with >20 ppm homogeneity over 4 cm [52]. Succinate was employed, because it finds potential application for cancer imaging [53–56]. With the improved DSV size and B_0 homogeneity, we report on *in vivo* high-resolution ^{13}C NMR spectroscopy capable of resolving splittings ~ 4 Hz arising due to ^{13}C - ^1H heteronuclear spin-spin couplings within the HP ^{13}C -SUX molecule (note that although ^{13}C - ^2H two- and three-bond couplings are present in HP ^{13}C -SUX (Figure 1), they are significantly weaker than the corresponding ^{13}C - ^1H spin-spin couplings, and they are likely manifested as line-broadening in the detected ^{13}C NMR spectra). We compare 48.7 mT ^{13}C *in vitro* and *in vivo* NMR spectra with those obtained at 4.7 T, and find at least one order of magnitude of ^{13}C linewidth (in the units of Hz) improvement at low magnetic field compared to high magnetic field.

Material and Methods

1.1. PHIP Hyperpolarizer

A PHIP hyperpolarizer used to prepare the 1- ^{13}C -succinate- d_2 hyperpolarized contrast agent (HCA) was fully automated via open-source software and hardware and integrated with a dual-channel low-field NMR spectrometer (Kea2, Magritek, Wellington, New Zealand), with operations controlled by computer through a graphical user interface (GUI) [52]. The Helmholtz saddle configuration radiofrequency (RF) coils used for the RF polarization transfer sequence developed by Goldman and Johannesson [57] operate at the ^1H and ^{13}C resonances for $B_0 = 5.75$ mT when capacitively tuned and matched to $50\ \Omega$ and enclose a high pressure plastic reactor with ~ 56 mL volume kept at temperatures between 50 – 60°C . Quality assurance of the produced agent before use is achieved via a small tipping angle RF pulse in order to establish the degree of polarization of the agent. The process of producing a dose of HCA takes less than a minute, and the routines for readying the system for the next dose, including cleaning, result in a total PHIP cycle time of less than 3 minutes. Full details of the PHIP polarizer instrumentation including schematics, software, and bill of materials are freely available elsewhere [52]. A home-built parahydrogen ($p\text{-H}_2$) generator using a low-power cryocooler is used to produce continuously 90% $p\text{-H}_2$ from bulk hydrogen at

flow rates up to 150 sscm and 25 bar pressure. A 1 L double-ended 316 SS sample cylinder with PTFE lining (316L-50DF4-500-T-PD, Swagelok) and brass Yor-Lok tube fittings (5272K291, McMaster-Carr) provides a gas reservoir for $p\text{-H}_2$ collection between experiments (~0.4 L is consumed per production of HCA, coincidentally matching conveniently the PHIP cycle time).

1.2. PHIP Hyperpolarization of 1- ^{13}C -succinate- d_2 (^{13}C -SUX)

Preparation of aqueous Rh-based hydrogenation catalyst and fumaric acid-1- ^{13}C - d_2 precursor used to produce HP succinate-1- ^{13}C - d_2 (^{13}C -SUX) followed previously the fully described previously procedure [52]. Briefly, precursor stock solution of fumaric acid-1- ^{13}C - d_2 (Cambridge Isotopes, CDLM-6062-PK, 1- ^{13}C 99%, 2,3- D_2 98%, 3.00 mmol, 0.357 g) is prepared in D_2O , degassed, pH adjusted to 10.3, and mixed with the standard PHIP catalyst/ligand system of rhodium(I) catalyst, bis(norbornadiene) rhodium(I) tetrafluoroborate (0.200 g, 0.54 mmol, 45-0230, CAS 36620-11-8, Strem Chemicals, MA) and phosphorus ligand, disodium salt of 1,4-bis[(phenyl-3-propanesulfonate)phosphine]butane (717347, Sigma-Aldrich-Isotec, 0.360 g, 0.64 mmol). Following preparation, the catalyst is stored in a plastic bottle attached to the gas/liquid manifold of the PHIP hyperpolarizer and kept under slight overpressure (~2–5 psi gauge pressure) of ultra-high purity nitrogen or Argon (>99.999%, A-L Compressed Gases Inc., Nashville, TN) [52].

1.3. MRI magnet and RF coils

The upright biplanar permanent magnet clinical imaging system (Supporting Information (SI) and Figure 2) generates B_0 field of 48.7 mT statically shimmed to ~13 ppm homogeneity over a 40 cm DSV (SIGWA 48.7 mT, Boston NMR, Boston, MA). The open-bore configuration achieves an 80 cm-gap between the planar XYZ gradient coil inserts mounted to the pole pieces. Heteronuclear NMR spectroscopic detection involving ^{13}C and ^{15}N on this system has been observed to approach ~1 Hz linewidths (determined as full width at half maximum or FWHM), when measuring NMR samples with μL volumes (*e.g.*, samples in 5 mm NMR tubes). We note that this system is particularly well suited for the direct NMR measurement of J -couplings of hyperpolarized compounds given its low field strength and excellent field homogeneity [30]. While not utilized for shimming in the reported *in vivo* study, when used for MRI the XYZ planar gradient coil set is capable of reaching 20 mT/m in all axes with linearity within approximately $\pm 6\%$ across the full FOV of 40 cm. Further details of the imaging system (including the Tecmag Redstone console) are provided in the SI.

The RF coil built for use in this imaging system was designed to measure metabolic HP contrast agents *in vivo* in small animal studies involving mice. The solenoid coil was constructed to match the typical dimensions of the animal (17 mm diameter by up to 193 mm body length) to increase the coil filling factor and correspondingly the SNR. The coil had a quality factor of 55. The coil was tuned using 56H02 Johansen trimmer capacitors and ATC nonmagnetic ceramic capacitors to tune the resonant frequency to ^{13}C resonance at 0.521 MHz or ^1H resonance at 2.074 MHz and match the coil impedance to the 50 Ω coaxial transmission line impedance. All component values are provided in Tables S1 and S2 in the SI. The resonant circuit for ^1H or ^{13}C detection was comprised of a parallel LC resonator

with a series capacitive matching element (see Figure S3 and S4, SI). The solenoid was constructed with 170 windings using 28 AWG gauge magnet wire with a measured inductance of 302 μH . The coil was enclosed in an RF shield constructed using square double-sided 1 oz. copper clad PCB materials (473–1007-ND, Digikey, Thief River Falls, MN) to form an enclosed cubic volume (1ft $\times$ 1ft $\times$ 1ft). This shielding provided an electric field suppression of 60+ dB. The coil was calibrated via a nutation sequence on the Redstone console using variable flip angle via pulse length calibration for fixed power, and the $\pi/2$ tip angle was determined to be 115 μs for ^1H and 475 μs for ^{13}C for <1 W of applied RF power. Further description of the RF probe is provided in the SI.

1.4. ^{13}C HP NMR spectroscopy and ^{13}C NMR spectroscopy using thermal polarization

Sodium 1- ^{13}C -acetate was used for Boltzmann thermal polarization analysis at 48.7 mT and 4.7 T for HP ^{13}C -SUX spectroscopic comparison given ~ 97 -fold field strength ratio for B_0 . HP ^{13}C -SUX spectroscopy was performed by injecting HCA into a 2.8 mL hollow plastic sphere situated inside the corresponding RF coil at either 4.7 T (best ^1H shim settings on the acetate phantom) or 48.7 mT (static magnet shim) followed by application of a small tipping angle hard RF pulse to observe the shimmed NMR resolution obtainable. For *in vivo* detection, a time series of NMR acquisitions to allow observation of HCA dynamics was commenced on the Redstone console at the start of the PHIP hyperpolarization sequence that produces a batch of HP ^{13}C -SUX.

1.5. ^{13}C spectroscopy in small rodents using HP ^{13}C -SUX

The feasibility of *in vivo* ^{13}C high-resolution detection of spin-spin couplings was tested in mice for HP ^{13}C -SUX at the low magnetic field of 48.7 mT. Procedurally, the 4T1 mammary carcinoma cells were maintained in RPMI-1640 medium supplemented with fetal bovine serum (10%, v/v) and the culture dish was incubated in a 37°C, 5% CO_2 . Eight-to ten-week-old female nude mice were purchased from Jackson Laboratory. All animal experimental protocols were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Nude mice 6–8 weeks of age (from Jackson Laboratory, Bar Harbor, ME, USA) were implanted sub-cutaneously under anesthesia (isoflurane mixed with 2% oxygen) with 1.0×10^6 cells in the mammary fat pad using a 27-G needle. The progress of tumor growth was monitored via every-other-day measurement of tumor size and animal weight. When the tumors reached approximately 4mm in diameter, *in vivo* ^{13}C spectroscopy study was performed.

In preparation for the NMR experiment, the mice were anesthetized using ketamine:xylazine (75mg/kg: 5mg/kg) via intraperitoneal injection. The anesthetic dose was designed to immobilize animals for approximately 60 minutes. After anesthetization, with the animal lying on a thin film carrier, a 28-gauge needle affixed to a catheter line was inserted into the lateral tail vein. The animal with catheter in place was then transported and placed inside the RF probe with the catheter line fitting through the RF shield. Prior to acquiring NMR spectroscopy or MRI imaging, animals were slowly injected over ~ 5 seconds timespan with ~ 0.2 mL of HP ^{13}C -SUX via the catheter in performance of a tail-vein injection. After NMR spectroscopy, animals were retrieved from the scanner and placed back into a warm cage under constant observation until recovered from anesthesia.

2. Results and Discussion

2.1. In vitro ^{13}C NMR Spectroscopy at 4.7 T and 48.7 mT

^{13}C NMR spectra of thermally polarized sodium acetate- $1\text{-}^{13}\text{C}$ (1 g dissolved in 2.8 mL D_2O DSV) were recorded using a 4.7 T Varian preclinical MRI scanner (Figure 1c, 1 scan) and using a Tecmag Redstone console (Figure 1c, 16 scans) at 50 MHz and 0.52 MHz ^{13}C resonance frequency, respectively. The high-field acquisition was performed using active shimming, while the low-field acquisition was performed without any additional magnet shimming. Acetate- $1\text{-}^{13}\text{C}$ moiety results in a characteristic ^{13}C quartet appearance due to ~ 6.0 Hz $^1\text{H}\text{-}^{13}\text{C}$ two-bond spin-spin coupling. The quartet features are somewhat better resolved at 48.7 mT, Figure 1c vs. 1f.

^{13}C spectroscopy of HP ^{13}C -SUX was performed at 4.7 T using the same type of hollow plastic spherical container. The resulting spectrum (Figure 1b) shows one relatively broad line with FWHM ~ 36 Hz. ^{13}C spectroscopy of HP ^{13}C -SUX performed at 48.7 mT was conducted in a plastic syringe container. The spectrum (Figure 1e) shows a very well resolved multiplet pattern—a manifestation of complex network of two- and three-bond $^{13}\text{C}\text{-}^1\text{H}$ spin-spin couplings. The half-height line width (HHLW) was estimated to be < 3 Hz, and the two outer peaks of the splitting pattern (~ 3.9 Hz away) are well differentiated. The ~ 12 -fold narrower linewidth (and consequently better resolution in units of Hz) obtained at 48.7 mT is possible due to the fact that susceptibility-induced field gradients which scale linearly with B_0 are significantly reduced at 48.7 mT compared to 4.7 T (by the ratio of ~ 97 of the two field strengths). The chemical shift dispersion (in units of Hz) scales linearly with B_0 , and as a result, it is reduced by a factor of ~ 97 at 48.7 mT compared to that at 4.7 T. The effective ~ 12 -fold line narrowing (due to reduced susceptibility-induced B_0 field gradients) described above partially mitigates the above reduction in chemical shift dispersion, and the overall resolving power of ^{13}C spectroscopy at 48.7 mT was approximately 8 times worse than that at 4.7 T (*i.e.*, $\sim 97:12$).

Despite nominal reduction of spectral resolution, the significant *in vivo* ^{13}C line narrowing at 48.7 mT vs. 4.7 T provides a certain advantage from the perspective of the detection sensitivity, or SNR. Because the sensitivity for HP detection can scale as $B_0^{1/4}$ [12] or as $B_0^{0.14}$ in the range of 10–500 kHz [58] depending on coil geometry and construction and the means of detection, which is a very weak field dependence, the significant reduction in FWHM is an important contributing factor to the overall detection sensitivity argument for low-field MR. In particular, this may translate into more sensitive LF MR detection schemes of HP contrast agents for some applications, which can take advantage of this significant narrowing of linewidth despite the commensurate reduction in the chemical shift dispersion, *e.g.* HP gas spectroscopy and imaging [5, 59].

2.2. In vitro ^{13}C -SUX T_1 in deoxygenated D_2O

Previous measurement of ^{13}C -SUX hyperpolarized lifetime in D_2O at 47.5 mT yielded 75 ± 3 s *in vitro* [15]. At 48.7 mT, the lifetime of HP ^{13}C -SUX in D_2O was measured to be 88 ± 5 s *in vitro* according to the decay curve shown in Figure 1 and accounting for using a RF pulse with flip angle (FA) $\sim 18^\circ$. This is in good agreement with previously reported value,

and the minor discrepancy is likely caused by slight imprecision ($< 1^\circ$) in calibrating the low-field RF pulse and small variations in the pH of the produced HCA, which is known to modulate ^{13}C T_1 of HP SUX significantly [53, 54, 60]. ^{13}C chemical shift anisotropy (CSA) decreases significantly in units of Hz at 48.7 mT compared to that at 3 T. As a result, ^{13}C CSA likely has a negligible contribution to the T_1 relaxation of the ^{13}C HP state at 48.7 mT, and dipole-dipole interactions likely represent the dominant T_1 relaxation mechanism at such low magnetic fields.

2.3. In vivo ^{13}C NMR Spectroscopy at 4.7 T and 48.7 mT

HP ^{13}C -SUX was injected as a bolus dose in the tail vein of anesthetized tumor-bearing mouse, which was positioned inside a RF coil placed in the center of 48.7 mT magnet (Figure 1). Non-localized ^{13}C NMR spectra were recorded via the NMR console commencing at the start of HCA production on the PHIP hyperpolarizer every 2 seconds using a small angle pulse for every RF excitation. The time window for recording NMR spectra of HP ^{13}C -SUX was ~80 seconds (Figure 2b). We note that the entire dynamic ^{13}C *in vivo* scan was effectively completed ~3 minutes after the beginning of contrast agent production in the PHIP hyperpolarizer. The ^{13}C NMR signal increases at the very beginning due to HCA entering the body of the animal, and it decreases after reaching a maximum post-injection due to (i) T_1 decay, (ii) RF pulse-associated losses, and (iii) possible metabolism. Despite the significant detection volume (whole mouse vs. a small syringe), the ^{13}C NMR linewidth remained largely unchanged: ~3 Hz (*in vivo*, Figure 2b) vs. ~3 Hz (*in vitro*, see above). While this result is not surprising because the susceptibility-induced gradients are negligible, the demonstration of such high-spectral-resolution HP ^{13}C low-field NMR spectroscopy *in vivo* has been performed for the first time to the best of our knowledge. Most *in vivo* preclinical experiments with ^{13}C HP contrast agents are performed at 3 T and above, and ^{13}C FWHM is typically 1 ppm corresponding to 30 Hz [55, 61–65]. The HP ^{13}C FWHM (in units of Hz) demonstrated here *in vivo* is at least an order of magnitude improvement compared to high-field *in vivo* studies of HP HCAs. Moreover, the FWHM of ~3 Hz (corresponding to ~6 ppm) is only a factor of 6 worse than that observed in high-field studies (*i.e.* 1 ppm) [55, 61–65]. While the chemical shift resolution (in units of ppm) is certainly reduced at 48.7 mT, this reduction is offset through the gain in sensitivity via ^{13}C line-narrowing, which represents an interesting trade-off between the detection sensitivity (*i.e.* SNR) and resolution.

2.4. Imaging and biomedical translation

Although HP NMR spectroscopy has been reported in the ZULF regime [37, 66, 67] including frequency-selective RF excitation [67], the use of conventional MRI frequency encoding in ZULF potentially is challenging due to the very low resonance frequency, and new MRI encoding techniques likely need to be developed. However, ^{13}C (as well as ^1H , ^3He and ^{129}Xe) MRI imaging can be performed at $B_0 > 6$ mT (including ~48.7 mT, the field studied here) as has been demonstrated by us [15, 52, 68–70] and others [8, 19, 20, 71–75] previously using conventional MRI approaches. In particular, ^{13}C frequency encoding can still be accomplished at ~0.5 MHz resonance frequency (f_0) as employed here and using a RF coil with suitable quality factor Q and reasonable choice of spectral width (SW) to satisfy the imaging bandwidth condition of $f_0/Q \gg \text{SW}$ [15]. The direct use of

conventional encoding approaches is a clear translational advantage of LF MRI compared to the current status of ZULF due to the ready availability of commercial hardware and the wide range of already established pulse sequences.

The ^{13}C NMR spectroscopy demonstrated here at 48.7 mT with ~6 ppm spectral resolution (~3 Hz FWHM) could be useful for *in vivo* ^{13}C spectroscopic studies aiming to: (i) differentiate HP pyruvate- ^{13}C and its main metabolic product in tumors of HP lactate-1- ^{13}C , because their HP ^{13}C resonances have a sufficient chemical shift difference of ~12 ppm [61, 62]; (ii) pH imaging via spectroscopic detection of HP ^{13}C -bicarbonate and $^{13}\text{CO}_2$, because their resonances are separated by ~35 ppm [76]; and (iii) potentially other injectable ^{13}C HP contrast agents with sufficiently different chemical shifts of HP metabolites [65, 77].

3. Conclusion

High-resolution (FWHM ~ 3 Hz) ^{13}C NMR spectroscopy of HP ^{13}C -SUX has been successfully demonstrated at 0.521 MHz resonance frequency *in vitro* and *in vivo* using a homogeneous (13 ppm over 40 cm DSV) 80 cm-gap 48.7 mT magnet. Resolution of ^{13}C line splittings of ~4 Hz were successfully observed *in vivo*. The *in vivo* ^{13}C linewidth (FWHM) obtained at 48.7 mT was ~12 times better than that achieved with a 4.7 T small-animal MRI scanner using the same HCA. A significant gain in spectral linewidth for ^{13}C low-field *in vivo* NMR spectroscopy is advantageous for improving the detection sensitivity of LF NMR. Potential biomedical applications can include monitoring HP ^{13}C agent uptake as well as detecting metabolism of biomolecules with sufficiently different chemical shifts at such low fields including MRSI of HP pyruvate-1- ^{13}C /lactate-1- ^{13}C , HP bicarbonate- ^{13}C / $^{13}\text{CO}_2$ and others [78–80].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Chemistry of ^{13}C -SUX hyperpolarization and NMR spectroscopy. (a) the conversion scheme of fumarate-1- ^{13}C -2,3- d_2 to yield succinate-1- ^{13}C -2,3- d_2 via the PHIP process. (b) and (e) ^{13}C NMR spectroscopy of HP ^{13}C -SUX in hollow plastic spheres (2.8 mL volume) at 4.7 T (best shim) and an HCA-filled syringe at 48.7 mT, respectively. (c) and (f) thermal Boltzmann polarization reference of 1 g sodium 1- ^{13}C -acetate in D_2O at 4.7 T (single average) and 5 g sodium 1- ^{13}C -acetate in 15 mL D_2O at 48.7 mT (16 averages), respectively. (d) T_1 decay curve of HP ^{13}C -SUX at 48.7 mT with simulated T_1 of 88 ± 5 s. See additional details in the text.

Figure 2.

Experimental setup and *in vivo* ¹³C NMR spectroscopy. a) Parahydrogen at ~80% purity was prepared with a home-built parahydrogen generator and used to produce HP ¹³C-SUX from ¹³C-FUM in a home-built PHIP hyperpolarizer. b) Coinciding with the start of production of HP ¹³C-SUX in the PHIP hyperpolarizer, NMR acquisitions every 2 s were acquired with FA=18°, with (insets) showing a selected NMR spectrum from the series alongside the corresponding SVD de-noised spectrum (10 largest singular values). See additional details in the text and SI.