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A Non-Synthetic Approach to Extending the Lifetime of Hyperpolarized Molecules using D₂O Solvation

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Abstract

Although dissolution dynamic nuclear polarization is a robust technique to significantly increase magnetic resonance signal, the short T_1 relaxation time of most ^{13}C -nuclei limits the timescale of hyperpolarized experiments. To address this issue, we have characterized a non-synthetic approach to extend the hyperpolarized lifetime of ^{13}C -nuclei in close proximity to solvent-exchangeable protons. Protons exhibit stronger dipolar relaxation than deuterium, so dissolving these compounds in D₂O to exchange labile protons with solvating deuterons results in longer-lived hyperpolarization of the ^{13}C -nucleus 2-bonds away. ^{13}C T_1 and T_2 times were longer in D₂O versus H₂O for all molecules in this study. This phenomenon can be utilized to improve hyperpolarized signal-to-noise ratio as a function of longer T_1 , and enhanced spectral and imaging resolution *via* longer T_2 .

Abstract

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Keywords

DNP; lifetime; T_1 ; T_2

1. Introduction

A major limitation to magnetic resonance imaging (MRI) is low sensitivity, resulting in long acquisition times.¹ Thus, most MRI acquisitions are restricted to endogenous ^1H -nuclei in water, taking advantage of the prevalence of water in biological tissues and the high natural abundance (99.98%) and sensitivity of ^1H , which scales with the nuclear gyromagnetic ratio (γ)². This is in stark contrast to the MRI-sensitive isotope of carbon, ^{13}C (natural abundance: 1.11%, $\gamma_{^{13}\text{C}}/\gamma_{^1\text{H}} \approx 1/4$). Nuclear hyperpolarization addresses the low sensitivity inherent to MRI and facilitates detection of non- ^1H nuclei by increasing the net alignment of nuclear spins with an external magnetic field. Dissolution dynamic nuclear polarization is the preferred method of hyperpolarization for biological applications, in which non-equilibrium nuclear spin populations are achieved through spin polarization transfer from unpaired electrons to atomic nuclei at $<1\text{K}$.^{3,4} Dissolution is performed after sufficient hyperpolarization is attained, during which superheated buffer dissolves and ejects the hyperpolarized (HP) compound for experimental use. This technique typically yields 10^5 -fold signal enhancement, facilitating real-time detection of biochemical processes on exogenously administered HP compounds. HP MRI has been applied to various pathologies^{5–7} but $[1-^{13}\text{C}]\text{-pyruvate}$ is the only clinically translated probe to date,^{8–10} partially due to the long spin-lattice relaxation time (T_1) of the carbon-1 resonance which dictates the HP lifetime.¹¹

Protium (^1H) is a source of dipolar relaxation^{12,13} and previous reports demonstrate that protium can drastically shorten $^{13}\text{C}/^{15}\text{N}$ T_1 relaxation times from up to 3-bonds away.^{14–19} Dipolar relaxation can be reduced by replacing protium with deuterium (^2H), usually involving the synthesis of deuterium labeled analogues that can be expensive, laborious, and impractical for most laboratories. Addressing this limitation, other groups have extended ^{15}N and ^{31}P T_1 times by exchanging directly-bound or 2-bond protons with deuterons by simply dissolving the compound in D_2O ,^{20,21} but these nuclei have limited applications in biochemical systems.

To facilitate *in vitro* and *in vivo* visualization of additional ^{13}C -labeled HP probes, our laboratory has characterized a non-synthetic method to significantly extend the T_1 and T_2 of ^{13}C -nuclei adjacent to nitrogen atoms with solvent-exchangeable protons, and we demonstrate that this technique can be used to improve *in vivo* SNR as a function of increased T_1 . Here, we replace solvent-exchangeable protons with deuterons and investigate its effect on ^{13}C -relaxation across 2-bonds. We expect the relative dipolar relaxation rate on ^{13}C to scale with γ ,¹⁹ and we hypothesize that this exchange creates an environment with weaker dipolar relaxation ($\gamma_{^1\text{H}}/\gamma_{^2\text{H}} \approx 6.5$) that increases the spin-lattice and spin-spin (T_2) relaxation times of nearby ^{13}C -nuclei.

2. Materials and Methods

Chemicals

[5-¹³C]-glutamine and [6-¹³C]-arginine were purchased from Cambridge Isotope Laboratories (USA), while [¹³C]-urea and [¹³C,¹⁵N₂]-urea were purchased from Sigma-Aldrich (USA). [5-¹³C,¹⁵N]-glutamine and [6-¹³C,¹⁵N₃]-arginine were synthesized in-house and the synthetic scheme was adapted from previously reported methods.^{14,22}

Buffers

14.1T thermal equilibrium measurements and hyperpolarized dissolutions were performed with a D₂O- or H₂O-based buffer. Each buffer consisted of 100mM Tris base and 1mM ethylenediaminetetraacetic acid (Fisher Scientific, USA). H₂O-based buffers were adjusted to pH 7.4 with conc. HCl and 10N NaOH (Fisher Scientific, USA) and D₂O-based buffers were adjusted to pD 7.4 with conc. DCl and 10N NaOD (Sigma-Aldrich, USA). pH and pD of H₂O- and D₂O-based buffers were measured with a Ag/AgCl electrode (Fisher Scientific, USA). Since the ionization constants of H₂O and D₂O (13.99 and 14.96)²³ and reduction potentials of H⁺ and D⁺ (0V and -0.044V)²⁴ are similar, we assumed that an Ag/AgCl pH electrode could be used to roughly approximate pD.

Thermal Equilibrium T_1 and T_2 Measurements at 14.1T

A 14.1T NMR spectrometer (Bruker, USA) was used to measure thermal-equilibrium ¹³C T_1 and T_2 values at 20°C. Samples were dissolved in either a 95:5 v/v mixture of the H₂O and D₂O-based buffers, or 100% of the D₂O-based buffer. 5% D₂O was added to the H₂O buffer to facilitate deuterium locking and minimize B₀ drift. Urea variants were dissolved to a final concentration of 100mM, whereas glutamine and arginine variants were dissolved to a final concentration of 50mM. T_1 values were measured using a standard inversion recovery sequence, and 15 different delay times between the π and $\pi/2$ pulses (spanning $3T_1$) were sampled. Each spectrum was an average of 3 to 4 scans, and a $5T_1$ to $6T_1$ pre-scan wait time was implemented to allow re-polarization of carbon nuclear spins between scans. T_2 values were measured with a standard Carr-Purcell-Meiboom-Gill (CPMG) sequence, $TR = 2\tau_{CPMG} = 20\text{ms}$ (or 10ms for [¹³C]-urea). At least 13 different total echo times spanning $3T_2$ were sampled. Each spectrum was an average of 2 to 6 scans, and a $5T_1$ to $6T_1$ pre-scan wait time was implemented to allow re-polarization of carbon nuclear spins between scans. For T_1 - and T_2 -relaxation curve fitting, the area under the curve (AUC) of the carbon resonance of interest was integrated for each spectrum. AUC values were plotted against time and fit to a mono-exponential recovery or decay function to calculate T_1 or T_2 , respectively.

Hyperpolarization of ¹³C-Enriched Molecules

To maximize polarization efficiency, all compounds in this study were dissolved in high molarity in the appropriate solvent to facilitate glass formation upon freezing. Each compound was prepared with a different formulation, as follows: Glutamine variants were mixed with 1.1 equivalent conc. HCl and dissolved to a final concentration of 1.5M using a 35:65 DMSO:H₂O (v/v) solution. Urea variants were dissolved to a final concentration of

6M in glycerol. Arginine HCl variants were mixed with 1 equivalent HCl and dissolved to a final concentration of 3.2M in H₂O. Each of these preparations contained OX063 radical (General Electric, USA) dissolved to a final concentration of 15mM. All samples were polarized in a SpinLab Polarizer (General Electric, USA) for at least 1h. Following polarization, the hyperpolarized substrate was ejected from the polarizer via dissolution with a large excess of superheated D₂O or H₂O-based buffer. The dissolution was collected in a pre-chilled glass flask (−20°C), which equilibrated to a final temperature of 25–35°C.

Hyperpolarized T_1 Measurements at 1T

Following polarization, 500μL of the hyperpolarized dissolution was added to an NMR tube and transferred to a 1T Spinsolve ¹³C NMR spectrometer (Magritek, NZ) as fast as possible, typically ranging between 10s–30s post-dissolution. For all compounds, NMR spectra were acquired every 3s with a 10° excitation over a timescale $>3T_1$. The area under the curve (AUC) of the carbon resonance of interest was integrated for each spectrum and plotted as a function of time, after which it was fit to a mono-exponential decay function (corrected for magnetization loss from each excitation pulse) to calculate T_1 . All reported measurements are averages of at least triplicate dissolutions, and the final concentration of the hyperpolarized compound following dissolution ranged from 5mM to 120mM. The substrate concentration in each dissolution was measured by ¹³C-NMR at 14.1T in the presence of 1 mM Gd-DOTA and a 10mM [¹³C]-bicarbonate or [¹³C]-urea standard. The integral of the resonance of interest was compared to that of the standard to calculate concentrations.

Hyperpolarized and Thermal Equilibrium T_2 Measurements at 1T

Thermal equilibrium and hyperpolarized CPMG-type T_2 was measured on [¹³C,¹⁵N₂]-urea at 1T with a Spinsolve ¹³C NMR spectrometer (Magritek, NZ) by a single echo train of on-resonance π pulses (66μs), following an initial $\pi/2$ pulse (33μs). Pulse lengths were measured on a 6M [1-¹³C]-acetate sample in H₂O, and shimming was performed on an H₂O sample. Signal was accrued for 2×1 ms between each pulse. The total inter-pulse delay was 4ms and the total number of pulses was 65536 for a ~260s acquisition time.

For hyperpolarized measurements, 200μl hyperpolarized [¹³C,¹⁵N₂]-urea was placed in a Shigemi tube immediately following dissolution to reduce errors due to B₁ inhomogeneity during CPMG acquisition. Dissolution concentrations ranged from 5–8mM [¹³C,¹⁵N₂]-urea. 3 experiments with hyperpolarized [¹³C,¹⁵N₂]-urea in D₂O and 2 experiments in H₂O were performed from single dissolutions. The first experiment for each dissolution was diluted 1:10 in the appropriate buffer to avoid oversaturation of the receiver. Fewer experiments were performed in H₂O due to the shorter T_1 of [¹³C,¹⁵N₂]-urea in H₂O. Decay curves were fit to a mono-exponential decay function to calculate T_2 . Discarding the first 1024 points to remove potential errors due to excitation of residual z-magnetization also revealed no significant effect in T_2 fit values.

In vivo Perfusion Imaging

All animal experiments were approved by the Institutional Animal Care and Use Committee at Memorial Sloan Kettering Cancer Center (protocol number 13–12–019). Two different samples of [¹³C,¹⁵N₂]-urea were prepared for polarization in a SpinLab polarizer, one for

dissolution with the D₂O-based buffer and the other with the H₂O based buffer. The D₂O and H₂O samples were polarized for 3h16m and 3h, respectively. Meanwhile, a 1/3mm diameter, 28cm long catheter (Braintree Scientific, USA) was placed in the lateral tail vein of a female Balb/c mouse (12 weeks old), and a 10U/mL heparin in saline solution was used to prevent coagulation and blockage of the catheter. The mouse was anesthetized with 1L/min room air, 1.5% isoflurane and loaded in a Bruker 3T MRI equipped with a dual tune ¹H/¹³C coil. A 6M ¹³C-urea phantom was placed besides the mouse, and the mouse was positioned such that its kidneys were centered within the coil. An axial ¹H *T_f*-weighted GRE containing the mouse kidneys and phantom was acquired on a 40×40mm field of view with 128×128 resolution and 2 mm slice thickness.

For hyperpolarized MRI experiments, the hyperpolarized urea dissolution was held above a small magnetic field (60G) for 25s following dissolution and prior to *in vivo* injection to simulate QC time. At 25s post dissolution, the mouse was injected with 100 μL of the dissolution (in addition to the 100 μL of dead volume in the catheter) over the course of 10 seconds. At the start of injection, an axial 2D ¹³C EPI pulse sequence was initiated. The acquisition was localized to the same region as the anatomic *T_f*-weighted image and acquired with a 36×36mm field of view (16×16 resolution), 10mm slice thickness, 15° excitation, 3s repetition time (40 repetitions), and ~5ms readout time for each time point. This procedure was performed with the D₂O-based dissolution, the mouse was kept in the MRI under anesthesia for 10 minutes, and the procedure was repeated with the second dissolution in H₂O on the same mouse.

[¹³C, ¹⁵N₂]-urea concentrations in each dissolution was measured by ¹³C NMR at 14.1T in the presence of a 20mM 2-¹³C-glycine standard and 1 mM Gd-DOTA. Concentrations were 27.6 mM [¹³C, ¹⁵N₂]-urea in H₂O, and 38.0 mM [¹³C, ¹⁵N₂]-urea in D₂O.

3. Results and Discussion

To test this hypothesis, we characterized the relaxation properties of [5-¹³C]-glutamine, [¹³C]-urea, and [6-¹³C]-arginine, which contain one, two, or three nitrogen residues covalently bonded to the *sp*² hybridized carbon, respectively (Figure 1). All reported *T_f* and *T₂* values were measured for the ¹³C-enriched position in H₂O or D₂O. Since ¹⁴N is a quadrupole nucleus (natural abundance: 99.63%, *I*=1), scalar and dipolar mechanisms both exhibit a dominant contribution to ¹³C relaxation times.²⁵ This is in contrast to ¹⁵N (*I*=½) for which scalar relaxation is significantly less pronounced, so ¹⁵N-enriched variants were included in this study to investigate the effect of scalar relaxation in both solvent systems.

Thermal equilibrium ¹³C *T_f* and *T₂* relaxation times at 14.1T were measured for all compounds in Figure 1 with a standard inversion recovery or Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, respectively (Table 1).¹² With respect to [¹³C]-urea, proton-to-deuteron exchange extends ¹³C *T_f* from 31.01s to 57.24s at 14.1T. Similar values and trends were measured for [¹³C, ¹⁵N₂]-urea, suggesting ¹⁴N-mediated scalar relaxation is not a dominant contribution to *T_f* relaxation at 14.1T (Figure 2A). Contrary to *T_f*, *T₂* times were sensitive to ¹⁵N-enrichment at 14.1T. ¹³C *T₂* only marginally increased for [¹³C]-urea when dissolved in D₂O, but *T₂* times were significantly longer for [¹³C, ¹⁵N₂]-urea in either

solvent. In addition, a large increase in T_2 was appreciated for [^{13}C , $^{15}\text{N}_2$]-urea when dissolved in D_2O , increasing from 20.12s to 34.61s (Figure 2B). The pronounced effect of ^{14}N -mediated scalar relaxation on ^{13}C T_2 and its negligible effect on T_1 has been previously demonstrated with urea at 3T.^{25,26} The field dependence of ^{14}N -mediated scalar relaxation on T_1 has also been reported,²⁵ as it is a dominant relaxation mechanism at near-zero field ($\sim 10\text{G}$) but becomes negligible at higher fields. The trends regarding ^{15}N -enrichment in Table 1 are consistent with previous results, but to our knowledge this has not been demonstrated in the setting of D_2O and H_2O solvation. Importantly, the trends observed for urea held for all compounds in this study and we postulate that this phenomenon can be generalized to other compounds with similar structures. In addition, though [^{13}C , $^{15}\text{N}_2$]-urea exhibits the greatest absolute increase in relaxation times when dissolved in D_2O , the arginine variants exhibits a similar or greater decrease in relaxation rate *via* D_2O solvation ($R_1 = 1/T_1$, $R_2 = 1/T_2$) when compared to urea. This is somewhat expected given that arginine has more exchangeable protons and since proton for deuteron exchange decreases dipolar relaxation rate as a function of decreased γ . However, this is a simplistic interpretation of the results and a thorough analysis of the relaxation mechanisms in each spin system is necessary to explain the change in relaxation rate between H_2O and D_2O solvation.

Though dipolar relaxation decreases *via* proton-to-deuteron exchange, scalar relaxation is introduced through the addition of deuterium ($I=1$). From Table 1, scalar relaxation at 14.1T can only be appreciated when measuring T_2 . Thus, we hypothesize that scalar relaxation from exchangeable deuterons is weak and does not offset the reduction in dipolar relaxation from proton-to-deuteron exchange, as solvation in D_2O increases T_2 for all compounds studied. This is reasonable considering scalar relaxation scales with J -coupling,¹² and we postulate that $^2J_{\text{CD}}$ is small since carbon-deuterium peak splitting was not apparent in our ^{13}C -spectra. In addition, $^2J_{\text{CH}}$ for covalently bound protons ranges from -10Hz to 15Hz ,¹² and we roughly approximate $^2J_{\text{CD}}$ falls within -1.5Hz to 2.3Hz since coupling scales with γ . $^2J_{\text{CD}}$ is likely even smaller for the compounds in this study due to rapid deuteron solvent exchange.²⁷ Therefore, we also expect minimal scalar relaxation from deuterium on ^{13}C T_1 at near-zero field, which is an important consideration for HP experiments since samples are briefly exposed to low magnetic fields in the moments following dissolution.

To test whether the thermal equilibrium trends at 14.1T also apply to the HP state, HP T_1 relaxation times were measured for these compounds at 1T. [$6\text{-}^{13}\text{C}$]-arginine in H_2O was omitted from this study because it contains three ^{14}N quadrupole nuclei that rapidly relax $6\text{-}^{13}\text{C}$ hyperpolarization in near-zero field immediately following dissolution, making accurate HP measurements challenging. The remaining compounds were polarized in a SpinLab (General Electric) and dissolution was performed using either buffered H_2O or D_2O . Pulse-and-acquire ^{13}C -NMR spectra were acquired with a 1T NMR spectrometer (Magritek) and fit to a mono-exponential decay function to calculate T_1 (Table 2)²⁸. Dissolution of the HP substrate in D_2O resulted in prolonged HP ^{13}C T_1 times at 1T, illustrated for [^{13}C , $^{15}\text{N}_2$]-urea in Figure 3A. ^{15}N -enrichment did not significantly affect HP T_1 values in either solvent for all compounds, similar to thermal equilibrium measurements at 14.1T, suggesting 1T is strong enough to mitigate scalar relaxation on T_1 . However, spectra for hyperpolarized compounds without ^{15}N -enrichment exhibited reduced SNR due

to decreased T_1 in near-zero field,²⁵ resulting in an increased rate polarization loss during transfer of the sample from the polarizer to the spectrometer. These spectra also exhibited increased line-broadening as a result of shorter T_2 at 1T. The T_1 increase in D₂O relative to H₂O ranged from 1.3 to 2.4-fold and loosely scaled with number of directly bonded nitrogen residues. Compared to other studies investigating deuterium-enrichment at 2-bond covalently bound hydrogen, our observed HP T_1 increase is comparable to or larger than that of other studies,^{14,17} though our measurements were recorded at a lower field strength. HP T_2 at 1T was also measured with a CPMG acquisition on [¹³C,¹⁵N₂]-urea, which increased from 32.6±0.8s in H₂O to 67.4±1.9s in D₂O (Figure 3B). These results demonstrate that this method can also be utilized to significantly extend HP T_2 , so long as the molecule is ¹⁵N-enriched to remove quadrupolar relaxation. Since these results are in line with the trends observed at 14.1T, we believe the HP T_2 at 1T of the other compounds also exhibit a similar trend.

All HP decay curves in D₂O exhibited mono-exponential behavior, implying proton-todeuteron exchange was complete by the time of signal acquisition (>15s post-dissolution). Proton exchange rates in H₂O for all compounds in this study have been previously measured, with urea and glutamine exhibiting the slowest exchange rates (~1s⁻¹).^{29–31} Since the concentration of all HP molecules following dissolution were 3–4 orders of magnitude lower than the deuterium concentration in D₂O (~10² M), we assume 1s⁻¹ is sufficiently fast to achieve isotopic equilibrium within a few seconds, as reflected in our data.

We also demonstrate that dissolution in D₂O can benefit *in vivo* experiments and facilitate clinical translation of new probes, despite the *in vivo* environment being primarily H₂O. A key difference between pre-clinical and clinical imaging studies is the addition of a quality control (QC) process. This delays administration of the HP solution by upwards of one minute, during which polarization decays as a function of T_1 .⁸ We hypothesized that dissolution in D₂O rather than H₂O can extend ¹³C T_1 during QC, translating to higher ¹³C polarization upon intravenous injection. [¹³C,¹⁵N₂]-urea was chosen for this proof-of-concept experiment, which has been extensively studied as a pre-clinical perfusion imaging agent and its prospects of clinical translation are promising.^{26,32–35}

Two samples of [¹³C,¹⁵N]-urea were polarized for ~3 hours and dissolved to similar final concentrations in either D₂O or H₂O. Dissolutions were held in a 60G field for 25s following dissolution to simulate QC and subsequently intravenously administered to a healthy mouse. At the start of injection, an axial 2D ¹³C echo-planar imaging (EPI) sequence localized to the kidneys was initiated with a 3s repetition time (Figure 4). Both dissolutions were injected into the same mouse, spaced 10 minutes apart, and no obvious morbidity was observed as a result of the injections. The images demonstrate that dissolution in D₂O increases HP signal in the kidneys, and the results reaffirm our hypothesis that deuterium mediated scalar relaxation is weak since the D₂O sample still exhibited higher signal than its H₂O counterpart despite being held at low field for 25s. In addition, assuming T_1 values at 60G are similar to 1T, the signal enhancement observed in Figure 4C is more than expected based on differences in T_1 relaxation during QC alone (which only yields a 33% SNR increase in D₂O after 25s). This implies a possible *in vivo* lengthening of the HP

state, suggesting D₂O is not immediately diluted into the blood and stays as a bolus with the HP substrate.

4. Conclusion

This study demonstrates that exchange of nitrogen-bound protons with deuterons by solvation in D₂O reduces dipolar relaxation on 2-bond ¹³C-nuclei at the cost of a minimal increase in scalar relaxation through the introduction of deuterium to the spin system. Given the dramatic increase in *T*₂ for ¹³C,¹⁵N-enriched molecules in D₂O (>300% in the case of arginine), higher spatial and spectral resolution can be acquired for *in vitro* HP experiments in D₂O. This method of improving *T*₁ and *T*₂ is not only limited to the three compounds in this study, but should be applicable to compounds with similar structures such as thiourea³⁶, amino acid derivatives,^{37–40} and small peptides,⁴¹ all previously studied with HP MR. However, the reported phenomenon appears to be somewhat specific for amide-like carbons, as less significant increases were observed for *sp*³ hybridized carbons in glucose when 2-bond hydroxyl protons are exchanged with deuterons.¹⁸ We hypothesize that bond lengths and angles play a role in mediating this effect, as the discussed relaxation mechanisms are *J*-coupling and distance dependent, and this will be investigated in future studies. Ultimately, this non-synthetic approach provides a means of extending the HP lifetime and has the potential to enhance *in vitro* biochemical assays and *in vivo* MRI. In future experiments, we intend to investigate whether *in vivo* *T*₁ and *T*₂ are indeed longer when HP compounds are administered with a D₂O bolus. *In vivo* lengthening of *T*₂ would be particularly interesting, as it will accommodate longer readout times and improve image resolution and signal intensity. As this method is independent of polarization technique, we believe this technique can be applied to the broader field of HP MR and used with PHIP-, SABER-, and optically-polarized molecules, so long as they are structurally similar to the compounds in this study.

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- Solvation of molecules with amide-like groups in D₂O extends amide-carbon T_1 & T_2 .
- This is due to a decrease in dipolar relaxation *via* proton to deuteron exchange.
- This phenomenon applies to the thermal equilibrium and hyperpolarized states.
- Decreased relaxation improves *in vivo* SNR in hyperpolarized MRI experiments.

Figure 1.
Chemical structures of all compounds included in this study.

Figure 2.

(A) Thermal equilibrium ¹³C inversion recovery (B) and CPMG decay curves at 14.1T, acquired on [¹³C]- and [¹³C, ¹⁵N₂]-urea in H₂O or D₂O, pH or pD 7.4, 20°C. (C) Nuclear spin quantum numbers (*I*) and gyromagnetic ratio ($[\gamma] = \text{MHz T}^{-1}$) of each nucleus in each urea variant, in H₂O and D₂O.

Figure 3. (A) 1D ^{13}C -NMR spectra (3s repetition, 10° pulse-angle, every 3rd spectrum shown) of HP $[\text{C}^{13}, \text{N}^{15}]$ -urea dissolved in H_2O or D_2O , pH or pD 7.4, 25–35°C. (B) T_2 decay curve for HP $[\text{C}^{13}, \text{N}^{15}]$ -urea in H_2O or D_2O at 25–35°C.

Figure 4.

(A) Axial ^1H T_1 -weighted gradient echo of a healthy female Balb/c mouse. Scale bar: 5mm.

(B) Axial ^{13}C -EPI with the same field of view (FOV) following injection of HP [^{13}C , $^{15}\text{N}_2$]-urea in H_2O or D_2O . Intensity values are the sum of the total intensity in time, normalized to the same scale. (C) Total ^{13}C signal in the FOV at each timepoint following injection with HP [^{13}C , $^{15}\text{N}_2$]-urea, normalized to [^{13}C , $^{15}\text{N}_2$]-urea concentration in each dissolution.

Table 1.¹³C T_1 and T_2 relaxation times at 14.1T

Compound	Labeling	T_1 (s)		T_2 (s)	
		H ₂ O	D ₂ O	H ₂ O	D ₂ O
Glutamine	¹³ C	10.66 ± 0.34	12.34 ± 0.21	0.354 ± 0.003 ^a	0.455 ± 0.004 ^a
Glutamine	¹³ C, ¹⁵ N	10.79 ± 0.34	12.29 ± 0.32	3.35 ± 0.21	4.66 ± 0.67
Urea	¹³ C	31.01 ± 1.18	57.24 ± 3.95	0.089 ± 0.001 ^a	0.100 ± 0.002 ^a
Urea	¹³ C, ¹⁵ N ₂	31.97 ± 1.38	57.34 ± 2.04	20.12 ± 0.17	34.61 ± 0.64
Arginine	¹³ C	6.62 ± 0.22	9.74 ± 0.32	0.184 ± 0.003 ^a	0.234 ± 0.004 ^a
Arginine	¹³ C, ¹⁵ N ₃	6.83 ± 0.19	9.71 ± 0.26	0.82 ± 0.02	2.68 ± 0.03

^a T_2 values of compounds without ¹⁵N-enrichment reported to 3 decimal points to demonstrate measurement accuracy.

Table 2.HP ^{13}C T_I relaxation times at 1T

Compound	Labeling	H_2O^a	D_2O^a
Glutamine	^{13}C	21.8 ± 1.8	34.1 ± 2.5
Glutamine	^{13}C , ^{15}N	23.6 ± 2.1	31.2 ± 3.8
Urea	^{13}C	51.6 ± 5.4	123.8 ± 19.6
Urea	^{13}C , $^{15}\text{N}_2$	53.3 ± 2.6	115.9 ± 8.2
Arginine	^{13}C	-----	32.1 ± 4.5
Arginine	^{13}C , $^{15}\text{N}_3$	13.3 ± 0.8	31.2 ± 6.8

^a T_I values reported in seconds as the average and standard deviation of at least 3 fits. Unpaired two-tailed *t*-test demonstrate that all T_I increases in D_2O were statistically significant ($P < 0.05$) except for [5- ^{13}C , ^{15}N]-glutamine ($P = 0.053$).