

Supplementary Material – Ultra-low field ^{13}C MRI of hyperpolarized pyruvate

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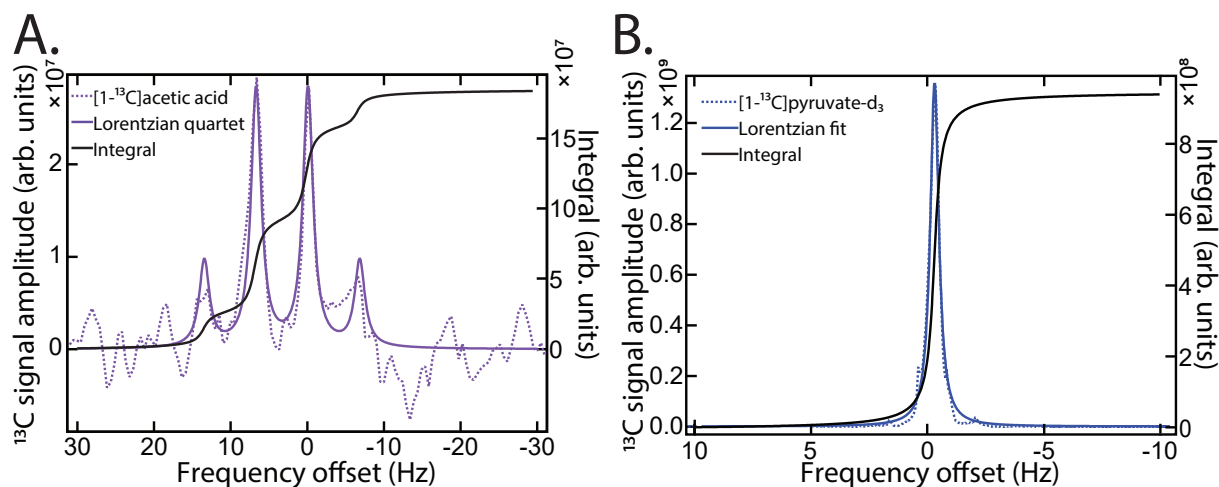
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Supplementary Section 1: ^{13}C polarization estimation

Thermal polarization of ^{13}C at 6.5 mT is too low to measure enhancement directly on 30 mM of ^{13}C enriched pyruvate so an estimation was performed by comparing the signal from thermal 1- ^{13}C enriched acetic acid as shown in Supplementary Figure 1. Acetic acid was selected because it is readily available in neat liquid form and not dissimilar in composition to pyruvate. The acetic acid spectrum is split by the 6.8 Hz J-coupling between the ^{13}C and methyl protons. (I) Acquiring 1024 averages over 4 hours 20 minutes, our signal-to-noise is sufficient to support constrained fitting of the anticipated quartet, even though the outer components are not clearly resolved. We fit the resulting data with a quartet of Lorentzian lines, holding the relative amplitudes to the expected 1:3:3:1 ratio and only using a single amplitude variable for fitting along with linewidth, J-coupling and chemical shift for a total of 4 fit parameters. The resulting fit gives a J-coupling of 6.76 ± 0.07 Hz, agreeing well with the expected splitting despite the poorly resolved outer lines of the quartet. Using a more concentrated ^{13}C -labeled compound, or a thermal reference acquired with a significantly higher number of averages, would provide a much more robust and reliable calibration of the thermal polarization. Knowing the molar concentration of ^{13}C in a 1.8 mL sample of neat 1- ^{13}C acetic acid, the gyromagnetic ratio, thermal Boltzmann polarization, and applying the Ernst equation with an estimated acetic acid T_1 of 40 s we calculated the enhancement in signal from hyperpolarized pyruvate compared to acetic acid to give the 3% estimate of ^{13}C polarization quoted in the main text. Matlab code used to calculate the estimate follows below. The ^{13}C polarization value for our estimate calculated by this procedure is 3.45%, but to appropriately reflect the substantial uncertainty associated with our estimate we only quote it to 1 significant figure.

```
signal_SABRE = 9.3907e8;
% signal_ace = 4.8494e7; %256 averages initial measurement 20250508
signal_ace = 1.8386e8; %1024 averages 20251217 for revision
averages = 1024;
theta = 45; % tip angle in degrees
TR = 15; % repetition time in s
T1 = 40; % estimated T1 in s
mass_pyruvate_2mL = 0.0065; %mass of pyruvate in g in 2mL
mass_pyruvate = mass_pyruvate_2mL/2*0.4; % " " in 400  $\mu\text{L}$ 
M_mass_pyruvate = 114.04; % molar mass [1- $^{13}\text{C}$ ]pyruvate-d3
M_pyruvate = mass_pyruvate/M_mass_pyruvate; % Moles of pyruvate
M_mass_ace = 61.04;
density_ace = 1.066;
M_ace = 1.8*density_ace/M_mass_ace; % Moles of acetic acid
molar_ratio = M_ace/M_pyruvate;

% calculate steady-state  $M_z$  after repeated pulses
M_zSS = (1-exp(-TR/T1))/(1-cosd(theta)*exp(-TR/T1));
```



Supplementary Figure 1: **Spectra for calculating an estimate of ^{13}C polarization at 6.5 mT.**

A. $[1-^{13}\text{C}]$ acetic acid spectrum acquired with 1024 averages of a 45° pulse every 15 s applied to a 31.4 mmol sample. A fit with the sum of four Lorentzians $y = (a/3)/((x-x_0)^2+b) + a/((x-x_0+J)^2+b) + a/((x-x_0+2J)^2+b) + (a/3)/((x-x_0+3J)^2+b)$ is plotted as a solid purple line and its integral plotted in black. **B.** SLIC SABRE hyperpolarized $[1-^{13}\text{C}]$ pyruvate- d_3 spectrum acquired in a single acquisition following 120 s SLIC SABRE with 24 sccm pH_2 flow through a sample containing 11.4 μmol pyruvate. A Lorentzian fit of the form $y = a/((x-x_0)^2+b)$ is plotted as a solid blue line and its integral plotted in black.

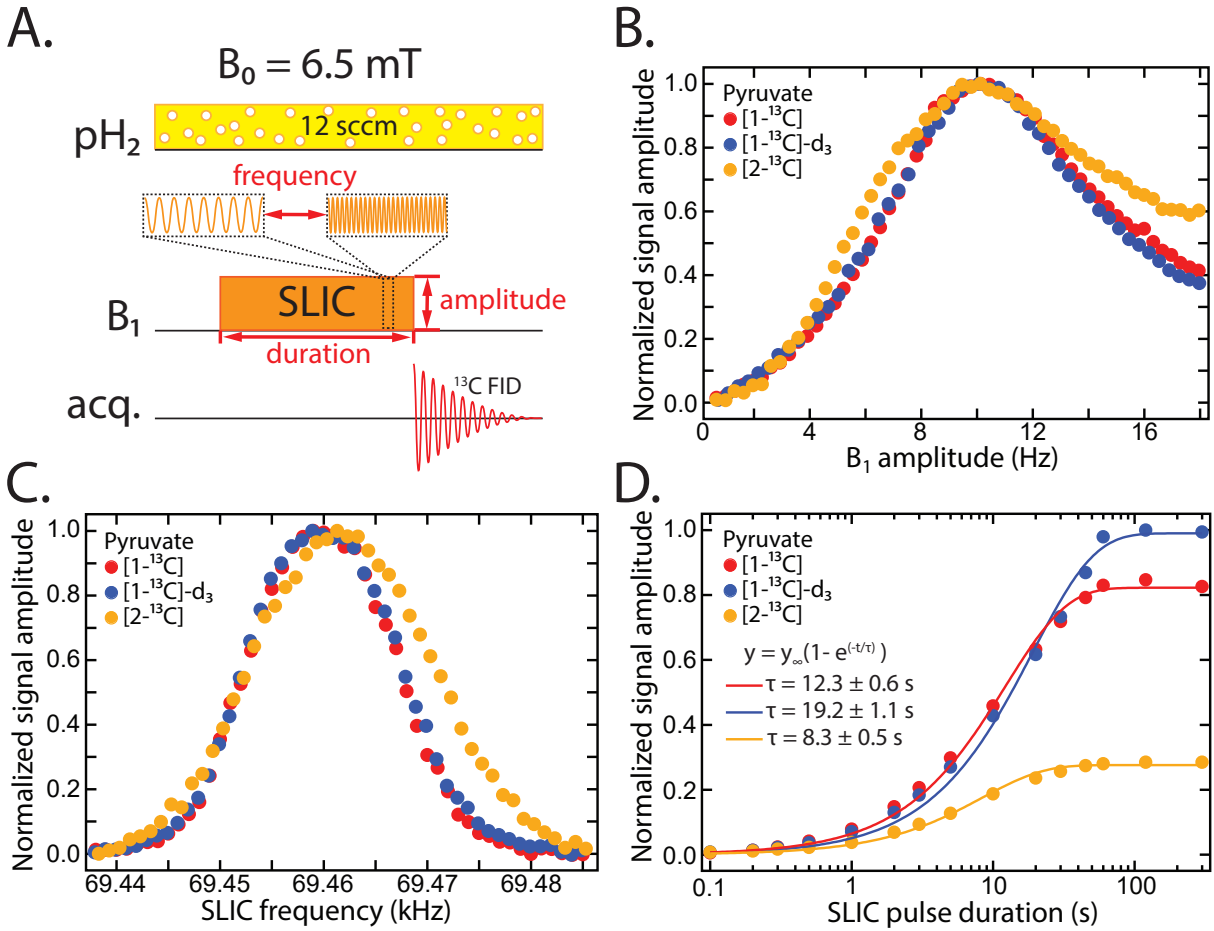
```
M_xySS = sind(theta)*M_zSS;
signal_ace_thermal90 = signal_ace/256 * (1/M_xySS);
signal_ace_thermal90_M = signal_ace_thermal90/M_ace;
signal_pyr_M = signal_SABRE/M_pyruvate;

B=6.5e-3; % Magnetic field strength in Tesla
T = 300; % Temperatures in K
gyro_13C=10.705e6; %gyromagnetic ratio for 13C in Hz/T/2pi
hbar=1.05457173e-34; %Planck's const. h/(2*pi)
k_B=1.3806488e-23; %Boltzmann's const.
thermal_P_13C=tanh((gyro_13C*hbar*2*pi*B)/(2*k_B*T))*100;

enhancement = signal_pyr_M / signal_ace_thermal90_M
13C_polarization = enhancement*thermal_P_13C
```

Supplementary Section 2: SLIC optimization

In this supplementary section, we turn to optimizing the parameters of the spin-lock pulse driving SLIC SABRE. The key advantages of performing in situ SABRE hyperpolarization and



Supplementary Figure 2: **Optimization of spin-lock properties for SLIC SABRE of ^{13}C -enriched pyruvate.** **A.** Pulse sequence diagram of a SLIC SABRE experiment showing the spin-lock variables investigated independently in B-D. **B.** Hyperpolarized ^{13}C signal as a function of spin-lock amplitude for protonated (red) and deuterated (blue) $[1-^{13}\text{C}]$ pyruvate and $[2-^{13}\text{C}]$ pyruvate (yellow). The most efficient transfer of spin order from pH_2 to ^{13}C occurs at $10.1 \pm 0.3 \text{ Hz}$. **C.** Transverse magnetization as a function of spin-lock frequency. Varying the RF frequency either side of the ^{13}C resonance at 69.46 kHz for $[1-^{13}\text{C}]$ pyruvate and 69.462 kHz for $[2-^{13}\text{C}]$ pyruvate leads to a reduction in the magnetization generated in the transverse plane. **D.** Build up of hyperpolarized ^{13}C magnetization with spin-lock duration. Data are fitted with an exponential function, plotted as a solid line, to quantitatively characterize build up rates. For all plots B-D, each data point is the integral of the pyruvate spectrum acquired following SLIC SABRE with the relevant independent variable altered from a standard experiment template of a 10 s spin-lock pulse on resonance, 10 Hz B_1 amplitude, 12 sccm pH_2 flow, and $4.4 \pm 0.2^\circ\text{C}$ sample temperature. For B and C, data are normalized to the maximum for each trace to aid comparison. For D, data are normalized to the deuterated pyruvate maximum to show the relative polarization achieved for each sample.

detection are: Firstly, that there is no need to transfer samples from an external polarizer to the MRI scanner and secondly, that the reversible chemical exchange in SABRE enables many experiments over time with the same sample. Experiments are reliably repeatable many times over using a single sample and negligible relaxation occurs between polarization and signal acquisition. We investigated how altering the frequency, amplitude and duration of the spin-lock affected the ^{13}C polarization achieved following SLIC SABRE as shown in Supplementary Figure 2A.

Supplementary Figure 2B shows the effect of tuning the amplitude of the spin-lock. The x-axis is plotted in terms of frequency experienced by spins in the rotating frame to allow for easy comparison to the relevant J-coupling, quoted in Hz rather than in μT of B_1 field strength as is more common for SABRE SHEATH. For the calibration method required to calculate the B_1 amplitude of a given output power from the spectrometer, refer to Supplementary Section 3. We find that the maximum occurs at 10.1 ± 0.3 Hz. Previously measured values for the J-coupling between hydride groups and the carbon in the 1st position on pyruvate bound to a SABRE catalyst molecule are $J_{HH} = -10.48$ Hz, $J_{HC} = 0.55, 0.014$ Hz (2) The combined hydride-carbon cross-coupling in the complex compares very closely with our measured optimum B_1 amplitude for SLIC SABRE. Similarly to the results for the SLIC frequency sweep, we note that the range of B_1 amplitudes that produce ^{13}C hyperpolarization is relatively broad, similarly explained by the efficiency of SLIC for magnetization transfer where the frequency difference between spin species is large. The $[2-^{13}\text{C}]$ pyruvate trace is slightly broader than for $[1-^{13}\text{C}]$ pyruvate. We attribute this to a combination of 2 effects: Firstly, the stronger J-coupling of the carbon in the 2 position to the methyl group alters the J-coupling network through which polarization flows from the hydrides on the SABRE catalyst. Secondly, after 10 s SLIC SABRE $[2-^{13}\text{C}]$ pyruvate has reached a higher fraction of its saturation polarization relative to $[1-^{13}\text{C}]$ pyruvate. This flattens the profile of the SLIC-amplitude vs enhancement curve as saturation effects begin to depress the maximum. This flattening effect occurs for $[1-^{13}\text{C}]$ pyruvate when equivalent experiments are run with longer SLIC pulses.

Supplementary Figure 2C plots how the ^{13}C signal acquired immediately following SLIC SABRE varies with the frequency of the spin-lock pulse. The maximum ^{13}C signal is acquired with the spin-lock on resonance with the ^{13}C Larmor frequency. The maxima occur at 69.460 kHz and 69.462 kHz for $[1-^{13}\text{C}]$ pyruvate and $[2-^{13}\text{C}]$ pyruvate respectively. The difference being due to chemical shift. As the SLIC frequency is varied either side of resonance the magnetization generated in the transverse plane decreases. If the spin-lock is only a few Hz off resonance, magnetization is also generated along the longitudinal axis. (3) While this process is less efficient, magnetization generated in this fashion can have the advantage that it decays with

T_1 rather than $T_{1\rho}$ or T_2 . This has been found to be a necessary method for generating bulk hyperpolarization in solution in cases where the chemical shift between free and bound pyruvate species means the bandwidth of the spin lock is insufficient to affect free pyruvate. One of the great advantages of performing SLIC SABRE at low field is that there is little chemical shift between bound and free pyruvate species. We note that the range of SLIC frequencies over which transverse magnetization is generated is much greater than both the few-Hz linewidth of the ^{13}C spectra and the sub-Hz spectral width of the 10 s spin-lock pulses. This occurs because SLIC efficiency is determined by the frequency difference between magnetically inequivalent spins. (4) Here, the comparatively large difference between the carbon spin in pyruvate and the proton singlet state on the SABRE catalyst lead to a SLIC SABRE hyperpolarization process that is robust to frequency offsets ± 10 Hz either side of the ^{13}C Larmor frequency.

The last SLIC pulse property to address is spin-lock duration, shown in Supplementary Figure 2D. As the spin-lock is applied, magnetization builds up in the transverse plane until it saturates at some level determined by the rate of pH_2 bubbling, chemical exchange, efficiency of polarization transfer and $T_{1\rho}$ relaxation. As discussed, SLIC polarization transfer is very efficient in this regime, and the pH_2 bubbling rate for these measurements was sufficient not to unduly restrict polarization, so the dominant processes determining the build up rate in this case are the pyruvate T_1 and chemical exchange rates. We find that for an equal pH_2 bubbling rate, deuterated pyruvate builds up to the highest polarization level while $[2\text{-}^{13}\text{C}]\text{pyruvate}$ polarization is comparatively modest. We attribute this difference to the longer T_1 of the deuterated pyruvate, allowing for a higher ^{13}C polarization to accumulate in the bulk solution before T_1 relaxation balances SLIC SABRE hyperpolarization. We draw the reader's attention to the speed of the hyperpolarization process in SLIC SABRE, emphasizing that these results are achieved close to room temperature and result in highly polarized samples in a matter of seconds. Compared to dissolution-DNP procedures this hyperpolarization process is more than an order of magnitude faster.

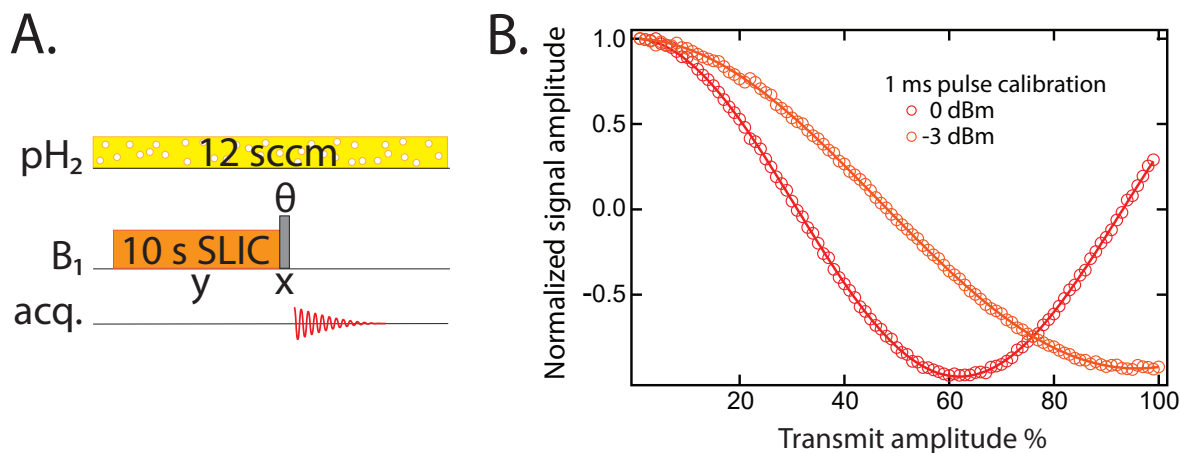
Supplementary Section 3: Pulse calibration and spin relaxation times

In this supplementary section we explain how we calibrate our B_1 amplitude before moving on to show results from experiments measuring spin-lattice (T_1) and spin-spin (T_2) relaxation times. To calibrate spin-lock amplitude, the quantitative relationship between nutation frequency and RF pulse power was determined in two steps: First, a rough calibration was

performed by ramping the electromagnet down to 1.6 mT to bring protons on resonance at 69.5 kHz. Measuring a high quality Rabi nutation curve on a CuSO_4 -doped water sample at this field is readily achievable within 10 minutes in our system with 16 averages of a simple pulse-acquire sequence for a 1 ms pulse length and a series of pulse amplitudes. Fitting the resulting curve with a decaying sinusoid function provides the pulse power calibration for protons, which can be used to calculate the corresponding values for ^{13}C at 6.5 mT by increasing the pulse power by the square of the ratio of the gyromagnetic ratios of ^1H and ^{13}C (12dB). This first calibration enabled spin-lock pulses of appropriate amplitude to be applied to SABRE samples to hyperpolarize pyruvate in situ via SLIC SABRE, giving sufficient ^{13}C signal at 6.5 mT for further calibration. The second calibration step was performed by measuring a nutation curve created by applying a hard RF pulse immediately following a spin-lock pulse that generated net ^{13}C magnetization in the transverse plane as shown in Supplementary Figure 3A. The resulting nutation curve, plotted in Supplementary Figure 3B, is in the functional form of a cosine as the hard pulse tips the net magnetization vector away from the transverse plane. Extracting the nutation frequency allows for the calculation of the strength of B_1 and calibration of pulse amplitudes. For example, a 90° pulse length of 1 ms, corresponds to a full 360° rotation in 4 ms or 250 Hz with 450 μW output from the console. From that calibration point, increasing the spectrometer output attenuation by 27 dB reduced the effective B_1 frequency to 11.1 Hz, and from there the transmit amplitude was adjusted to perform B_1 amplitude sweeps such as those shown in Supplementary Figure 2B. We note that this calibration method assumes the spectrometer transmit amplitude adjustment is linear and the attenuation values are accurate.

Having demonstrated that we have reliable tip angle calibration for hard pulses we move on to the investigation of spin-lattice relaxation. For in vivo hyperpolarized imaging applications, spin-lattice relaxation time (T_1) is critical because it determines how long the hyperpolarized signal remains visible and how far down the chain of metabolic products information can be extracted. Much of the success of pyruvate as a hyperpolarized metabolite is due to its comparatively long T_1 at tesla field strengths: $[1\text{-}^{13}\text{C}]\text{pyruvate}$ and $[1\text{-}^{13}\text{C}]\text{pyruvate-d}_3$ T_1 times are approximately 2 and 4 minutes at 2 T. (5)

Pyruvate T_1 decay curves measured at 6.5 mT are shown in Supplementary Figure 4. Our pulse sequence for measuring T_1 in a SLIC SABRE experiment is drawn in Supplementary Figure 4A. The typical SLIC SABRE pulse sequence was modified by the addition of 2 hard 90° pulses. As SLIC SABRE on resonance produces net magnetization aligned with B_1 in the transverse plane of the Bloch sphere, to measure T_1 relaxation the magnetization is rotated to lie along the longitudinal axis by the first 90° pulse. After some evolution time, the magnetization is then returned to the transverse plane for readout by the application of another 90° pulse



Supplementary Figure 3: **Pulse calibration with magnetization generated by SLIC SABRE.**

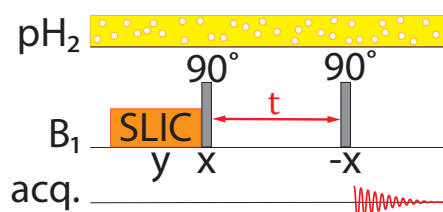
A. Pulse sequence for secondary tip-angle calibration. Following the application of SLIC SABRE to generate transverse ¹³C magnetization in a pyruvate SABRE solution, a hard pulse θ is applied with orthogonal phase to the spin-lock pulse, rotating magnetization out of the transverse plane. **B.** Repeating the pulse sequence in A, varying the spectrometer transmit amplitude for 1 ms pulses with output power of 0 dBm (red) or -3 dBm (orange), results in nutation curves with the functional form of a decaying cosine (plotted as solid lines). The transmit amplitude corresponding to a 90° pulse occurs at the first zero crossing.

with the opposite RF phase and an FID acquired. The resulting decay curves were fitted with a decaying exponential of the form $y = e^{-t/T_1}$ where t is the time between the hard 90° pulses and T_1 is the spin-lattice relaxation time.

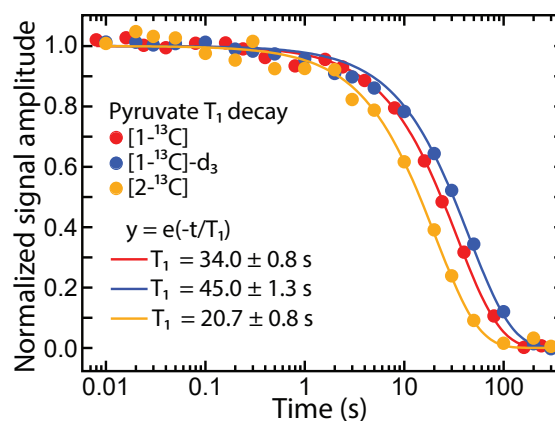
The resulting data are plotted in Supplementary Figure 4B and fitted to a decaying exponential function to extract the characteristic T_1 decay constant. We measure T_1 values of 34.0 ± 0.8 s, 20.7 ± 1.3 s and 45.0 ± 1.3 s for $[1\text{-}^{13}\text{C}]$ pyruvate, $[2\text{-}^{13}\text{C}]$ pyruvate and $[1\text{-}^{13}\text{C}]$ pyruvate- d_3 respectively. These relaxation times are consistent with an interpolation from previous results measured at microtesla and tesla fields. (5) We note that these times are measured while the sample is undergoing constant pH_2 bubbling action that continues during the delay in our T_1 measurement sequence. The ongoing bubbling may introduce convection, susceptibility fluctuations, and residual SABRE-related polarization effects, potentially influencing the measured relaxation time. This does not appear to significantly affect T_1 relaxation as our results are comparable to experiments using small tip angle sequences on pyruvate samples hyperpolarized outside the 6.5 mT scanner and transferred inside for measurement, shown in Supplementary Figure 5. However, there is reason to believe that given there are ongoing SABRE processes occurring, including proton hyperpolarization at 6.5 mT, our T_1 values measured here should be regarded as the "effective T_1 " specific to these conditions.

With a measure of T_1 established for our system, we consider T_2 relaxation. The variable tip angle schedule used by our SLIC-shot bSSFP sequence is determined by the choice of initial tip, the number of pulses, T_1 , T_R and T_2 . (7) To determine an appropriate T_2 value for calculating our tip angle schedule we used a sequence similar to a Hahn echo, as shown in Supplementary Figure 6A. The typical Hahn echo experiment was modified by the removal of the initial 90° pulse as the SLIC SABRE spin-lock generates transverse magnetization. Bubbling was continued throughout, matching our other measurements. Results from this Hahn-echo-style measurement performed on $[1\text{-}^{13}\text{C}]$ pyruvate- d_3 are plotted in Supplementary Figure 6B and fitted with Gaussian and exponential decay functions of the form $y = e^{-(t/T_2)^2}$ and $y = e^{-t/T_2}$ respectively, where t is the time between the end of the spin-lock and the center of the spin echo, and T_2 is the spin-spin relaxation time. The mono-exponential decay appears insufficient to describe all the features of the observed behavior, but gives a good representative value to inform our variable tip angle schedule. Given the echo time used in our SLIC-shot MRI experiments was 54 ms, much less than our T_2 of ~ 2 s, our variable tip angle schedule was relatively insensitive to T_2 compared to T_1 and our choices of initial tip angle and number of lines of k-space to acquire. As with our experiments measuring T_1 relaxation, the constant bubbling occurring throughout our Hahn-echo-style experiments likely influences the measured relaxation time, which should similarly be considered an "effective T_2 " rather than an intrinsic

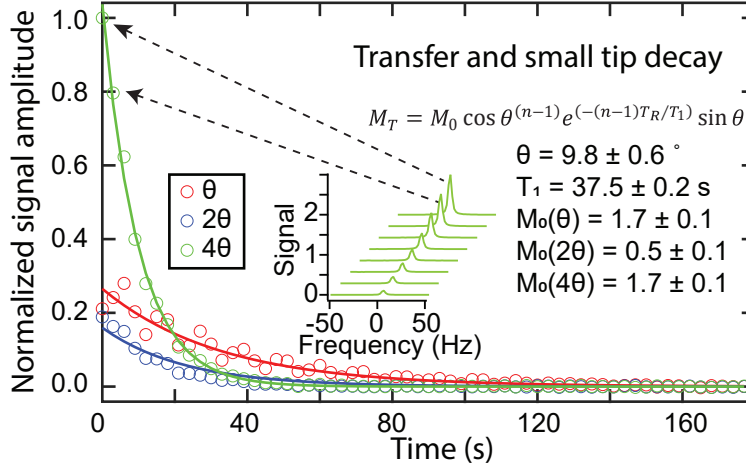
A.



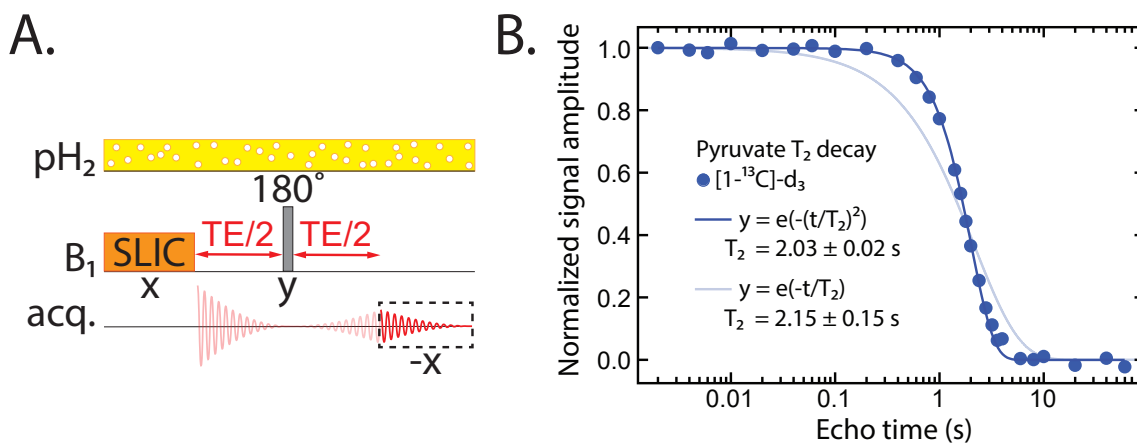
B.



Supplementary Figure 4: **Spin-lattice relaxation of hyperpolarized [1-¹³C]pyruvate.** **A.** Pulse sequence for measuring T_1 decay of SLIC SABRE hyperpolarized pyruvate. Following 10 s SLIC SABRE on resonance at 69.46 kHz, at 10 Hz B_1 amplitude, ¹³C magnetization was rotated out of the transverse plane to lie along the longitudinal axis by a hard 90° pulse, before being returned to the transverse plane by another hard 90° to measure T_1 relaxation of the hyperpolarized state. pH₂ flow was held constant at 12 sccm and temperature was held at $4.4 \pm 0.2^\circ\text{C}$. **B.** Measured hyperpolarization decay curves. Data points are integrals of [1-¹³C]pyruvate (red), [2-¹³C]pyruvate (yellow) and [1-¹³C]pyruvate-d₃ (blue) spectra. Solid lines are exponential fits.



Supplementary Figure 5: **Small tip angle measurements of hyperpolarization decay.** [1-¹³C]pyruvate hyperpolarized outside the 6.5 mT scanner with a SLIC SABRE polarizer (described in reference (6)) and transferred to 6.5 mT for measurement. Following shuttling of a hyperpolarized ¹³C sample into the scanner, a pulse sequence of 60 RF pulses, spaced 3 s apart, was applied and the FID acquired with a solenoid coil tuned and matched to 69.5kHz. For subsequent experiments the tip angle was incremented by doubling the pulse amplitude. As the 3 small tip angle decay curves then have a common T_1 and known relationship between their applied tip angles, they can be simultaneously fitted to 3 variations of the equation $M_T = M_0 \cos \theta^{(n-1)} e^{-(n-1)T_R/T_1} \sin \theta$, where M_T is the transverse magnetization acquired following the n th tip, M_0 is the initial magnetization delivered for each run, θ is the tip angle, and T_R is the repetition time of 3 s.



Supplementary Figure 6: **Spin-spin relaxation of hyperpolarized $[1-^{13}\text{C}]$ pyruvate- d_3 .** **A.** Pulse sequence for measuring T_2 decay of SLIC SABRE hyperpolarized pyruvate. Following 30 s SLIC SABRE on resonance, ^{13}C magnetization dephases in the transverse plane before a 180° pulse is applied with orthogonal phase to the initial spin-lock to refocus the magnetization into a spin echo. To keep the acquisition length consistent across all echo times only the second half of the spin echo is acquired. pH_2 flow was held constant at 30 sccm and temperature was held at $4.4 \pm 0.2^\circ\text{C}$. **B.** Measured spin-echo decay under constant bubbling conditions. Data points are integrals of $[1-^{13}\text{C}]$ pyruvate- d_3 spectra, solid lines are exponential (light blue) and Gaussian (dark blue) fits with corresponding extracted decay constants.

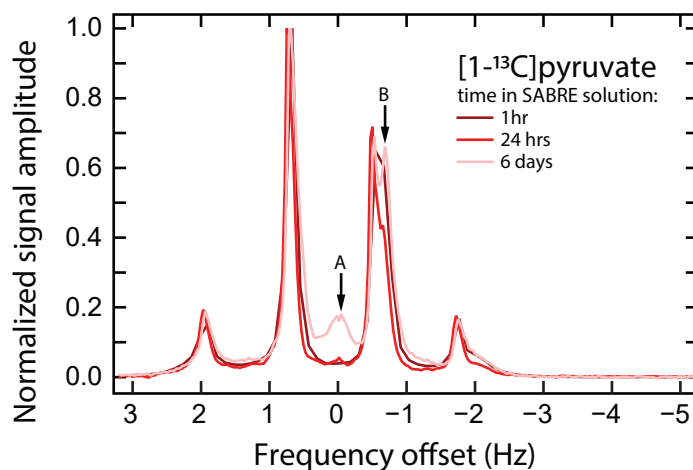
sic relaxation constant for our SABRE solutions in the millitesla regime. The apparent non-mono-exponential behavior observed in our Hahn-echo experiments is probably caused by the magnetic susceptibility fluctuations from ongoing bubbling. While a spin-echo experiment can refocus field inhomogeneities over timescales slower than the echo time, at the 30 sccm pH_2 flow rate used for this measurement, bubbling creates local fluctuations in magnetic field on much faster timescales than the relevant echo time regime. Spin dephasing from these fluctuations is not refocused by the echo experiment, resulting in a steeper decay at these timescales than described by the mono-exponential curve usually observed in liquid-state NMR. Our data are better described by a Gaussian function, usually associated with decay curves from Hahn-echo experiments where inhomogeneous broadening dominates spin-spin relaxation.

Supplementary Section 4: Further discussion of fine features in pyruvate spectra

The fine details in our $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ pyruvate- d_3 spectra shown in Figure 4 of the main text include peaks in the wings of the deuterated pyruvate spectrum and a peak in the middle of the quartet in the protonated pyruvate spectrum. Extending the discussion of spectral features in the main text, here we explain why we attribute their origin to the exchange of protons and deuterons, and describe how these additional enigmatic features evolve over time in SABRE solutions. While secondary to the main narrative of our manuscript we share these measurements as useful validation of the need to prepare a fresh batch of sample for each SABRE experiment. They are also of interest as they reinforce evidence that SABRE cannot always be rigorously considered a non-hydrogenative parahydrogen induced polarization process. (8) Further, to investigate other potential explanations we show 11.7 T NMR spectroscopy data from SABRE solutions after their use in SLIC SABRE experiments at 6.5 mT.

For the simple case of $[1-^{13}\text{C}]$ pyruvate- d_3 , the quartet of peaks about the primary signal are most likely due to imperfect deuteration of the methyl group. The quartet of peaks matches the $[1-^{13}\text{C}]$ pyruvate quartet and the provider-quoted deuterium fraction is $\geq 97\%$. While this explanation is straightforward, it also offers a clue to help explain our observations for protonated pyruvate: The unexpected central feature in the $[1-^{13}\text{C}]$ pyruvate spectrum occurs at the same frequency as the main $[1-^{13}\text{C}]$ pyruvate- d_3 peak, suggesting a common origin. This central feature also increases in magnitude over time, as shown in Supplementary Figure 7, which is consistent with deuteration occurring, mediated by the SABRE catalyst, with the methanol- d_4 solvent providing an abundant source of deuterium.

High-field NMR spectra measured at 11.7 T are shown in Supplementary Figure 8, comparing $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ pyruvate- d_3 ^1H and ^{13}C spectra from samples following SLIC SABRE experiments. These samples were stored at room temperature for several hours, likely accelerating any processes at work in the data shown in Supplementary Figure 7. No significant variation can be identified between samples. There are no spectral features sufficiently near the pyruvate peak to suggest an impurity may account for our observations at 6.5 mT. At 6.5 mT, the peak at about 176 ppm in the high-field ^{13}C spectra would be seen in the hyperpolarized 6.5 mT spectrum +1.7 Hz away from pyruvate, and the peak around 181 ppm would be at about +3 Hz. Neither peak appears in the SLIC-SABRE hyperpolarized spectrum, suggesting these species are not hyperpolarized under our SLIC SABRE conditions.



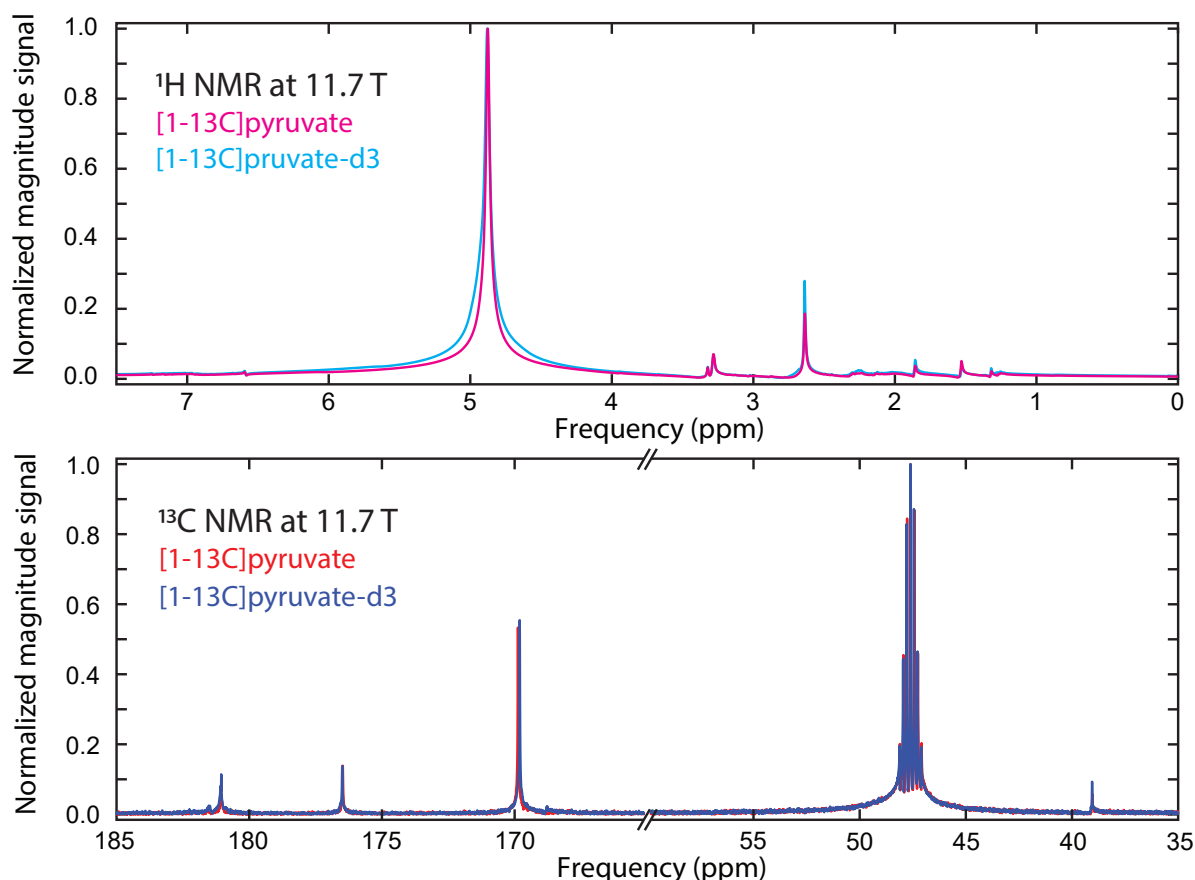
Supplementary Figure 7: **[1-¹³C]pyruvate ¹³C spectral evolution.** A single batch of SABRE solution was prepared, stored refrigerated under argon, and sampled for SLIC SABRE experiments at 6.5 mT at 3 time points. Arrows draw attention to features at "A" and "B" that are not explained by the quartet expected from [1-¹³C]pyruvate. We attribute feature A to deuterated pyruvate, and feature B to free [2-¹³C]pyruvate, likely present in small concentrations from imperfect enrichment.

Supplementary Section 5: Optimizing conditions for SABRE

This supplementary section considers the optimum pH₂ flow rate and temperature conditions for maximizing pyruvate polarization at 6.5 mT. These results are most important in the context of informing future polarizer and reactor design. For completeness, we also briefly present our pH₂ generator and describe how our gas handling and temperature control circuits interface with the SLIC SABRE experiment situated inside the 6.5 mT scanner.

The gas handling circuit used to supply pH₂ to the SABRE solution inside the RF-shielded scan room is shown in Supplementary Figure 9A. Before an experiment begins the gas lines are pumped and flushed with pH₂. Bypass valves built into the circuit aid this process and enable pH₂ bottle and SABRE sample swaps without necessitating depressurizing all gas lines. A purge valve inside the scan room acts as a test point to confirm hydrogen delivery and helps leak checking by providing a true positive reading location. To bubble pH₂ through the SABRE solution the pressure regulator on the pH₂ supply bottle is set to 110 psi and the back pressure regulator set to 95 psi. The mass flow controller (MFC) can then throttle pH₂ independently.

For experiments investigating the effect of varying pH₂ flow rate, SLIC pulses were applied on resonance, with 10 Hz amplitude and 30 s duration. After each adjustment of the MFC flow



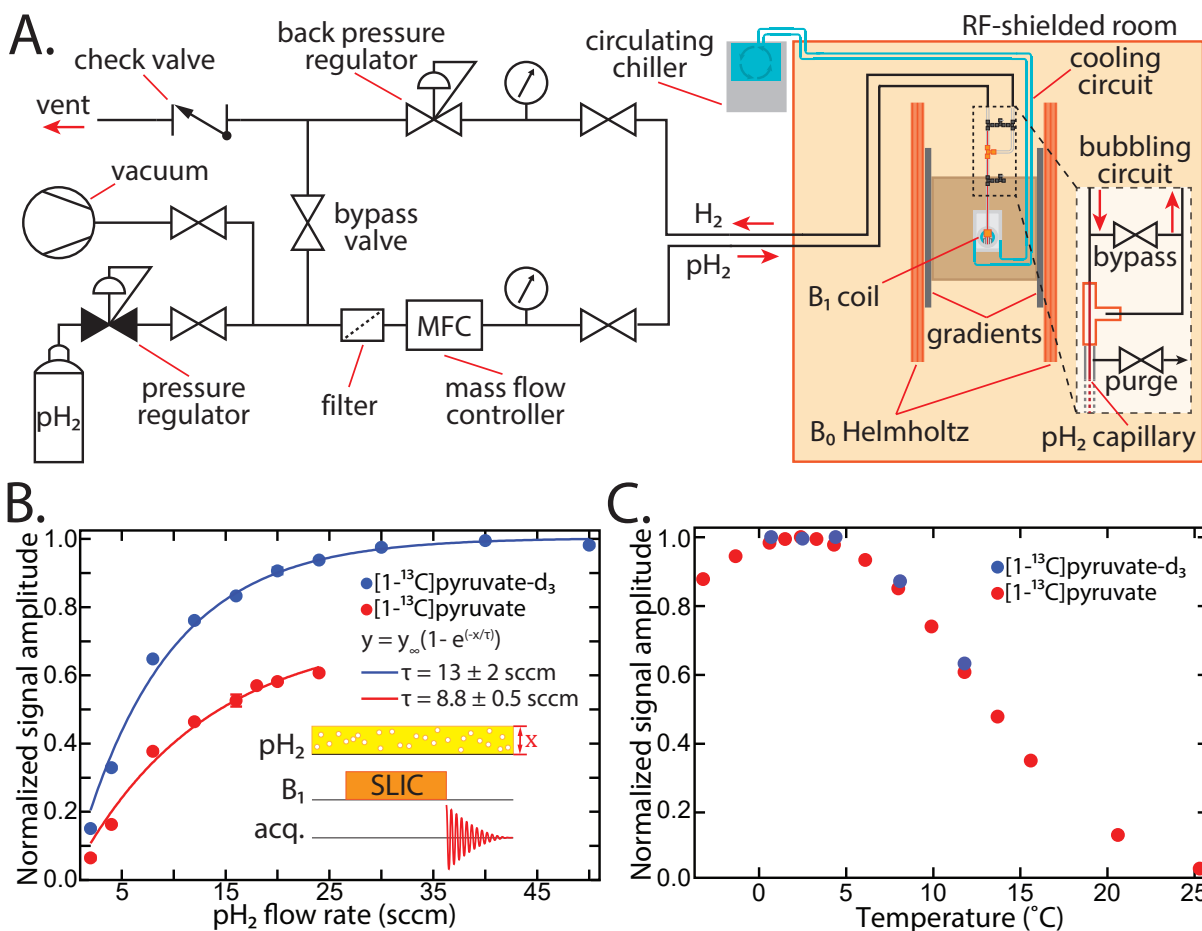
Supplementary Figure 8: **NMR spectra of pyruvate SABRE solutions at 11.7 T following SLIC SABRE experiments at 6.5 mT. Top:** ¹H NMR of [1-¹³C]pyruvate (magenta) and [1-¹³C]pyruvate-d₃ (cyan). ¹H spectra are dominated by a broad hydroxyl group feature. **Bottom:** ¹³C NMR of [1-¹³C]pyruvate (red) and [1-¹³C]pyruvate-d₃ (blue). Methanol-d₄ accounts for the largest feature – a septet from the deuterated methyl group – with the second largest feature around 170 ppm the [1-¹³C]pyruvate peak. We attribute the feature at approximately 176 ppm to pyruvate hydrate and suggest the small peaks around 181 ppm may be zymonic acid or para-pyruvate. (9) Spectra were measured with a JEOL 500 MHz NMR console with the frequency axis shown here calculated from the spectrometer offset calibration rather than an independent reference.

setting, multiple consecutive SLIC SABRE experiments were performed to verify the polarization achieved had reached repeatable equilibrium at the new flow rate. This usually occurred within the first 2–30 s repetitions. Once polarization levels had stabilized at a new flow setting, results were averaged and the standard deviation used to quantify the uncertainty. Build up curves were fitted with an exponential of the form $y = 1 - e^{-x/\tau}$ where x was the pH_2 flow rate and τ the build up constant.

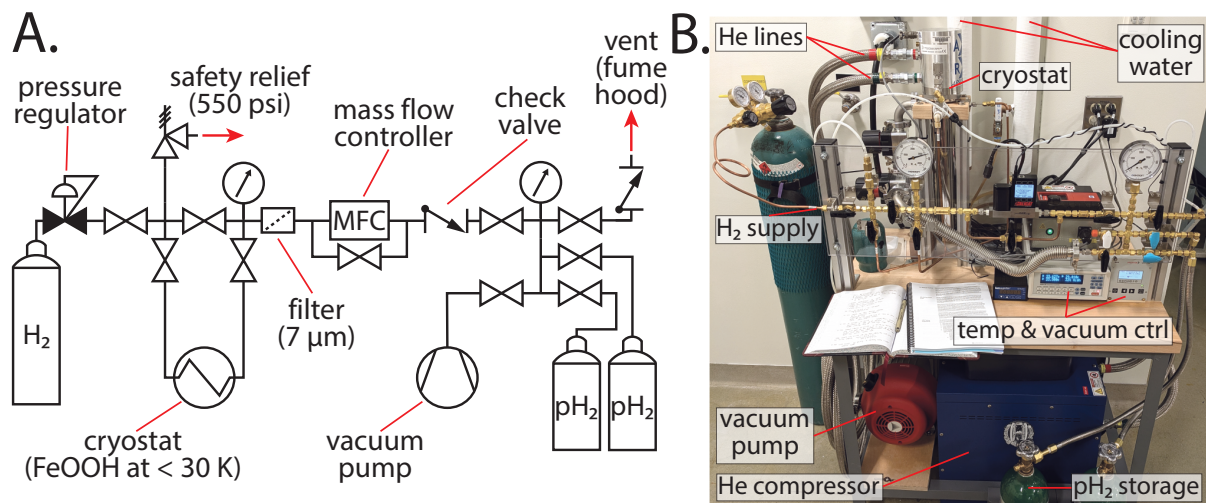
To investigate the temperature dependence of SLIC SABRE at 6.5 mT the same basic experiment for investigating pH_2 flow rate was employed, but instead of varying MFC setting, the chiller setpoint was changed and the sample temperature allowed to re-equilibrate before each set of measurements, while pH_2 was held constant at 12 sccm. For each setpoint, the temperature was stable to within $\pm 0.2^\circ\text{C}$, and 4 averages were acquired with 30 s SLIC on resonance. The standard deviation between each set of four measurements was used to estimate uncertainty.

Results plotting the signal enhancement as a function of pH_2 flow rate are presented in Supplementary Figure 9B. Each point plots the ^{13}C signal acquired immediately following 30 s SLIC SABRE on resonance. Exponential curves are plotted as a guide to the build up rate rather than to represent an analytical solution to the competing processes that govern these experiments. The behavior here is dominated by the fluid dynamics of bubbling through a small capillary into the bottom of a narrow tube. With ideal uniform diffusion of pH_2 through the solution, saturation would occur when pH_2 is supplied fast enough to ensure every H_2 that binds to a catalyst molecule is statistically likely to be fresh pH_2 rather than oH_2 . However, in our case saturation occurs when bubbling forces the sample out of the NMR coil. This effect is already pronounced at 24 sccm and has been foreshadowed by the ^{13}C MRI results in Figure 3 showing hyperpolarized solution being pushed upwards. This highlights the need for optimized polarizer designs and the benefit of reactors that control the distribution of hydrogen gas into solution. (10, 11)

Supplementary Figure 9C plots the SLIC SABRE signal as a function of temperature for 1- ^{13}C pyruvate. Each data point is 4 averages following 30 s SLIC on resonance with pH_2 bubbling at 12 sccm. The protonated and deuterated pyruvate samples exhibit the same temperature dependence, with maximum polarization achieved at $2.5 \pm 1^\circ\text{C}$. Varying the temperature of the SABRE solution modulates the chemical exchange rate of pyruvate molecules binding and releasing the catalyst. At low temperature, pyruvate binds too strongly to the SABRE catalyst, slowing chemical exchange and constricting the generation of bulk hyperpolarization of free pyruvate solution. At higher temperatures, pyruvate exchanges too rapidly for polarization transfer to occur mediated through J-coupling between the parahydrogen singlet state and pyruvate across the SABRE catalyst. While our results demonstrate that there is a clear opti-



Supplementary Figure 9: **Parahydrogen and temperature control** **A.** Schematic representation of the gas handling and cooling circuits used to perform SLIC SABRE experiments inside the 6.5 mT MRI scanner. **B.** ^{13}C hyperpolarization build up vs pH_2 flow rate for deuterated (blue) and protonated (red) $[1-^{13}\text{C}]\text{pyruvate}$ at 6.5 mT. Insert shows simplified pulse sequence emphasizing the variation in the rate of pH_2 bubbling characterized here. Data are normalized by division by the maximum signal from the deuterated pyruvate. Data are fitted with an exponential function, plotted as a solid line, to quantitatively characterize build up rates. Each data point is the integral of four averages of the pyruvate spectrum acquired following 30 s SLIC SABRE on resonance at 69.46 kHz, 10 Hz B_1 amplitude, with sample temperature held at $4.4 \pm 0.2^{\circ}\text{C}$ and pH_2 pressure at 95 psi. Error bars are the standard deviation, plotted when they are larger than the size of the data point. **C.** Hyperpolarized ^{13}C signal as a function of temperature. Data points are the integrals of hyperpolarized spectra, measured with 4 averages of 30 s SLIC on resonance at 12 sccm pH_2 . Data normalized to maximum signal for each pyruvate sample. Error bars are smaller than data points.



Supplementary Figure 10: **pH_2 generator.** **A.** H_2 gas handling circuit schematic. Only the section of the vacuum system for pumping H_2 is shown. **B.** Labeled photograph of the pH_2 generator in operation. The gas handling circuit is mounted on a transparent polycarbonate panel in front of the cryostat, with the compressor, vacuum pump and pH_2 cylinders underneath.

imum temperature, we observe a broad temperature range of $>10^\circ\text{C}$ where at least 80% of the maximum enhancement is produced. This shows that the temperature control requirements for efficient SABRE hyperpolarization in future polarizer designs are less strenuous than those for efficient pH_2 delivery, homogeneous B_0 and B_1 fields and rapid sample purification.

Finally, we briefly share the details our pH_2 generator, shown in Supplementary Figure 10. The pH_2 generator consists of a closed cycle cryostat (ARS, DE-204S cold head) driven by a helium compressor (ARS-4HW) and monitored by a temperature controller (Lakeshore, LS335). A scroll pump (Pfeiffer, HiScroll12) serves to evacuate the cryostat vacuum shroud before generator operation and pump contaminants out of the H_2 gas handling circuit. The cryostat sample chamber is packed with FeOOH catalyst, sieved to $> 45\mu\text{m}$, contained with quartz wool (McMaster-Carr) and filter paper (Whatman, grade 602h) to prevent migration through the H_2 circuit. During operation, the cryostat temperature is set to 27 K and 500 psi H_2 gas is flowed through the catalyst-packed sample chamber, with flow rate maintained at 2 slm by a mass flow controller (Alicat, MCQ-5SLPM). Aluminium cylinders are pumped and flushed with fresh pH_2 before filling to 480 psi. Check and bypass valves act to prevent flow through the generator of any contaminants introduced during cylinder swapping. All hydrogen that is released during operation, via the vacuum pump, vent valve, or 550 psi relief valve, is vented into a fume hood.

Supplementary Section 6: SLIC SABRE at mT vs T and μ T

Compared to SLIC SABRE performed at high magnetic fields, the millitesla regime has three key advantages: Firstly, pH_2 bubbling can be applied continuously without magnetic susceptibility effects causing significant distortion in the B_0 and B_1 fields. Secondly, there is no singlet-to-triplet mixing of parahydrogen. (12) Thirdly, chemical shift effects are small enough that the singlet state between hydride groups is maintained without spin locking and the ^{13}C Larmor frequency for bound and free pyruvate species overlap sufficiently for the SLIC pulse to spin-lock free pyruvate spins in the transverse plane following hyperpolarization. Overcoming these effects requires the additional complications of RF pulses to spin-lock protons and selective RF pulses to excite free and bound spins. (13–15) These challenges have limited the pursuit of in situ SLIC SABRE hyperpolarization for ^{13}C MRI at high-field, despite the potential of in-situ setups to provide fast, low-cost hyperpolarization platforms (avoiding the need for a separate polarizer device) and help visualize reaction dynamics to evaluate performance and inform reactor design. These benefits of in-situ hyperpolarized MRI have spurred PHIP experiments (16–18) which offer a viewpoint from which to reflect upon our work. The hyperpolarized ^{13}C MRI achieved to date with this in-situ method imaged a pyruvate PHIP-SAH precursor molecule inside a 7 T preclinical scanner. (18) Our results compare favorably, for although preclinical gradients used in Ref (18) enable sub-second experiments with sub-mm in-plane resolution, image signal to noise is comparable, if not inferior to our SLIC-shot MRI results despite our approximately ten-fold lower polarization starting point.

Comparing SLIC SABRE at millitesla fields to microtesla experiments, the crucial advantage is that conventional inductive detection is still practical and there is no need to resort to complicated superconducting magnetometer setups for readout. (19) As a consequence, compared to the best recent pyruvate ^{13}C MRI in the μT regime, our SLIC-shot MRI results achieve comparable resolution and SNR within less than 1% of the imaging time (including both the hyperpolarization and MRI acquisition steps). (10)

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