Supplementary Information

Identification of Intra- and Extracellular Metabolites in Cancer Cells Using ^{13}C Hyperpolarized Ultrafast Laplace NMR

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Table of Contents:

1. Supporting Figures and Tables for Experimental Section

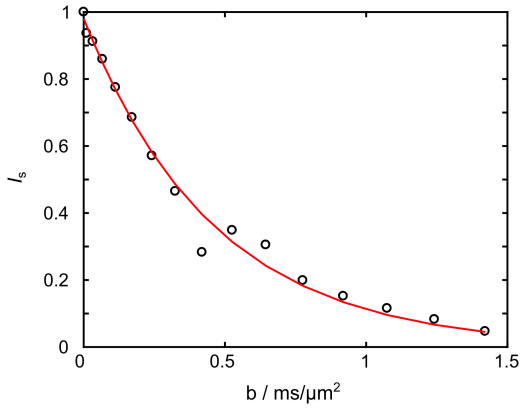

Figure S-1. ^{1}H NMR diffusion signal of pyruvate on its methyl proton at 308 K as a function of $b = (\gamma G\delta)^2(\Delta - \delta/3)$, using a conventional PGSTE sequence. The spectrum was acquired under the same sample condition after the *D*-T_2 experiment of hyperpolarized pyruvate selection without cell suspension. The final pyruvate concentration is 13.5 mM. The red fitting line yields a self-diffusion coefficient of D = (2.2 ± 0.4) · 10^{-9} m^2/s.

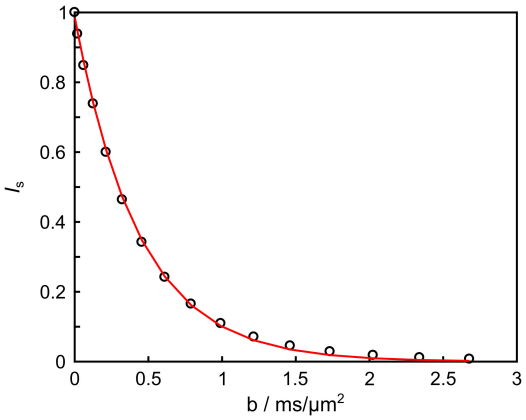

Figure S-2. ^{1}H NMR diffusion signal of H_2O at 25°C as a function of $b = (\gamma G\delta)^2(\Delta - \delta/3)$, using a conventional PGSTE sequence. The fitted curve using $I_s = I_0 \cdot exp(-b \cdot D)$ is shown in red. Using a self-diffusion coefficient of D_{water} = 2.3·10^{-9} m^2/s from published values,[1] the z-gradient strength G was calibrated as 6.5 G/mm.

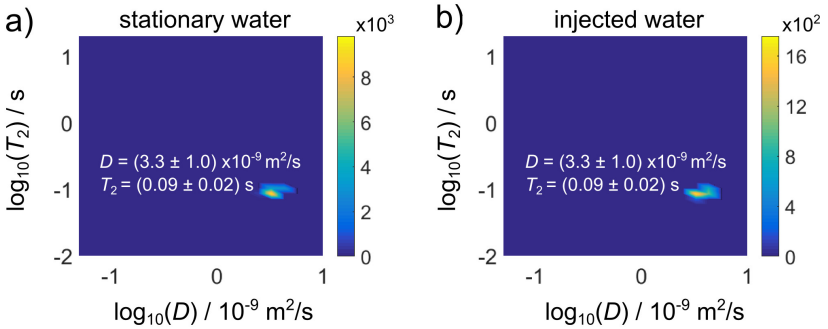

Figure S-3. a) *D*-T_2 map of stationary water in the flow cell at 308 K. D value agrees well with published value which is 2.9 × 10^{-9} m^2/s at 308 K.[1] b) *D*-T_2 map of water injected into the flow cell. 8 µL of water was loaded into the DNP polarizer and transferred to the flow cell without hyperpolarization. Injection procedures were the same as the actual hyperpolarized *D*-T_2 measurement. The measurement was triggered after the 20 s waiting time. The final temperature after injection is 308 K.

Table S-1. Experimental parameters for D-T_2 measurement of thermally polarized water

π/2 Gaussian pulse duration	2122 μs
π/2 Gaussian pulse excitation bandwidth	996 Hz
t_{chirp}	2.0 ms
chirp pulse bandwidth	34.1 kHz
chirp pulse $(\gamma B_{1max})/2\pi$	3.7 kHz
π pulse (CPMG loop) $(\gamma B_1)/2\pi$	29.1 kHz
G_{diff}	10.7 G/cm
$G_{dephase}$	7.8 G/cm
G_{read}	7.8 G/cm
$t_{G,diff}$	4.0 ms
$t_{G,dephase}$	4.1 ms
$t_{G,read}$	7.2 ms
Δ	100 ms
t_{CPMG}	[0.02 s, 1.28 s] with interval 0.02 s
δ_{eff}	[0 ms, 4 ms]
points in the T_2 dimension	64
complex points in the diffusion dimension	256
spectral width	100 kHz
scan number for stationary water	5
d1 for stationary water	5 s
t_{exp} for stationary water	33 s
scan number for injected water	1
t_{exp} for injected water	2 s

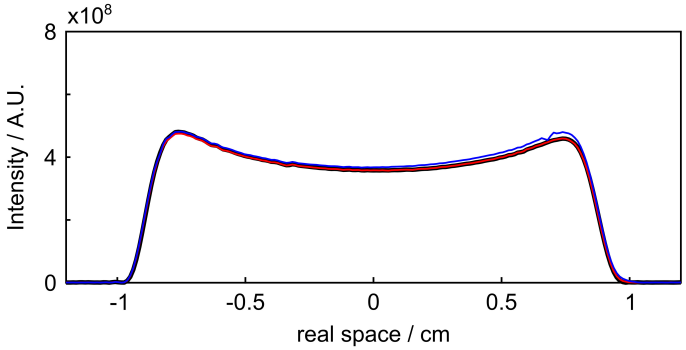

Figure S-4. Coil image profiles. 8 µL water was loaded into the DNP polarizer and injected using the same procedures as for the actual hyperpolarized *D*-T_2 measurement. A first measurement were triggered after a waiting time of 10 s (blue curve), and a second measurement after 15 s (red curve). As a reference, an image was obtained by acquiring signal of static water in the flow cell (black curve).

2. Additional Data with Selection of Lactate Signal in Cell Suspension

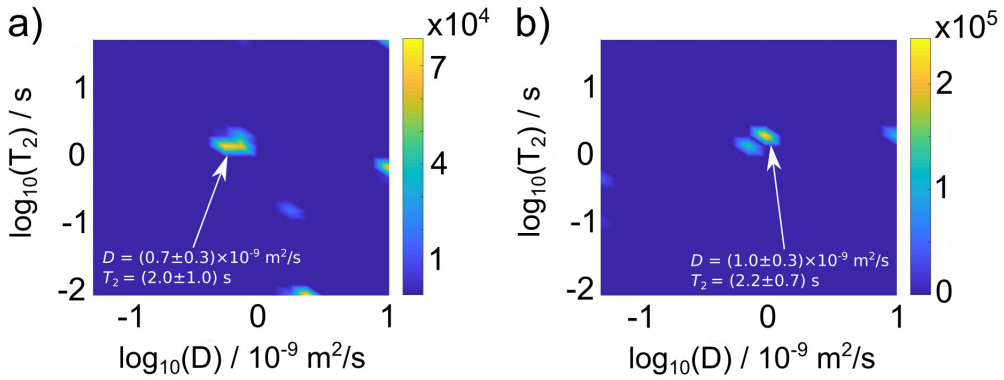

Figure S-5. a) and b) are chemically selective ultrafast *D*-T_2 maps of lactate in cell suspension. These two data sets were measured under the same conditions as the data shown in Figure 3c. The *D* and T_2 values shown in the figures correspond to the maxima of the peaks. The uncertainties are estimated from the width of the peaks.

3. Calculation of T_2 Relaxation Parameters

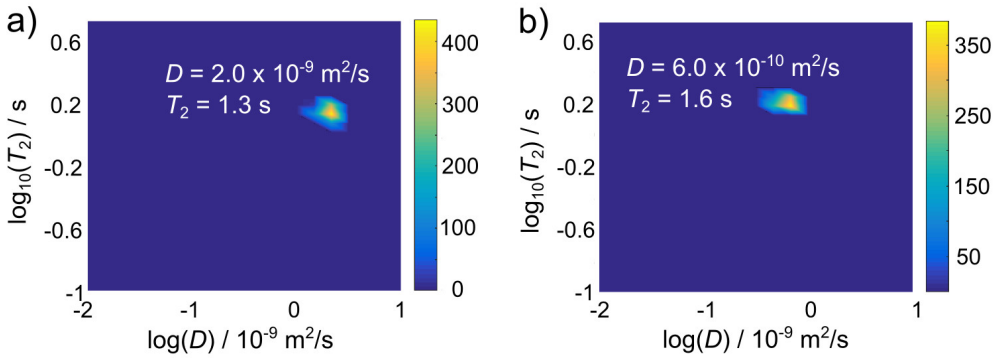

Figure S-6. a) Simulated D-T_2 map of molecule A with $D = 2.0 \cdot 10^{-9}$ m^2/s. b) Simulated D-T_2 map of molecule B with $D = 6.0 \cdot 10^{-10}$ m^2/s. Relaxation time T_2', which is observed in the absence of a gradient, is 1.7 s for both A and B.

The simulation of D-T_2 data are described elsewhere.[2] Briefly, the echo amplitudes are calculated using the equation: $E(k,l) = S_{max} exp[-D\gamma^2 \delta_{eff}(k)^2 G_{diff}{}^2 \Delta] exp[-t(l)(-1/T_2{}' - \beta)]$, where S_{max} is the signal amplitude, D is diffusion coefficient, δ_{eff} [0 ms, 5 ms] is effective length of G_{diff}, G_{diff} = 36.4 G/cm is gradient strength, Δ = 50 ms is diffusion delay, and T_2' is relaxation time that would be observed in the absence of a gradient, $\beta = (8\gamma^2 G_{read}{}^2 te^2 D)/\pi^4$ is the diffusive attenuation factor, which further depends on echo time of the first echo te = 20 ms and G_{read} = 2.3 G/cm. Therefore, depending on the relative magnitude of the changes in β and T_2', the observed T_2 can increase or decrease. All parameter settings, as indicated, are the same as in the experiment. For the simulation, data was generated using the above equation in Matlab. Normally distributed noise was added using matlab function "randn". The simulated data was then processed using the same method as the experimental data in Figure 3.

4. Analysis of Single *D* and T_2 Traces

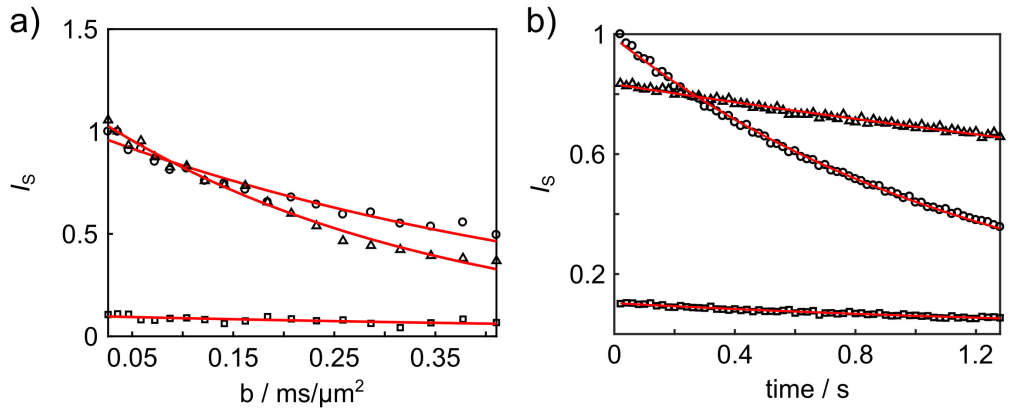

Figure S-7. a) Signals from the 8th columns of hyperpolarized *D*-T_2 data, shown in the diffusion dimension as a function of $b = (\gamma G_{diff}\delta_{eff})^2(\Delta - \delta_{eff}/3)$. Triangle, circle and square symbols stand for hyperpolarized *D*-T_2 data of pyruvate selection without cell suspension, pyruvate selection with cell suspension and lactate selection with cell suspension, respectively. Signals are normalized to the maximum signal amplitude of hyperpolarized *D*-T_2 data set with circle symbols. Fitted curves using the Stejskal–Tanner signal equation $I_s = I_0 \cdot exp(-b \cdot D)$ are shown in red. The fitting results are *D* = (3.0 ± 0.1) · 10^{-9} m^2/s, (1.9 ± 0.4) · 10^{-9} m^2/s and (1.2 ± 0.6) · 10^{-9} m^2/s for curves with triangle, circle and square symbols. b) Integral of each hyperpolarized *D*-T_2 data in the diffusion dimension is shown as a function of t_{CPMG}. Symbols represent the same data sets shown in (a). The integral comprises the range shown in (a). Signals are normalized to the maximum integral with shortest t_{CPMG}. Integrals are fitted with $I_s = I_0 \cdot exp(-t/T_2)$. The fitting results are T_2 = (5.4 ± 0.2) s, (1.2 ± 0.0) s and (1.9 ± 0.3) s for curves with triangle, circle and square symbols.

Under the assumption that there is only one signal component in the hyperpolarized UF *D*-T_2 data sets, the Laplace inversions can be compared to single *D* or T_2 traces from the same data set. The Fourier transform of hyperpolarized *D*-T_2 data in each echo leads to 64 columns along the T_2 dimension, corresponding to 64 diffusion curves (Figure S-8). Signals from the 8th columns of hyperpolarized data are plotted in Figure S-7a as a function of *b* values. Fitting diffusion curves of each data set results in mean *D* values as shown in Figure S-8. T_2 values are derived by Fourier transform and integration of hyperpolarized *D*-T_2 data in the diffusion dimension (Figure S-7b). As shown in Figure 3, the *D* and T_2 values of the *D*-T_2 maps match those from the individual trace analysis.

5. Diffusion Coefficient Fitting of 64 Echos

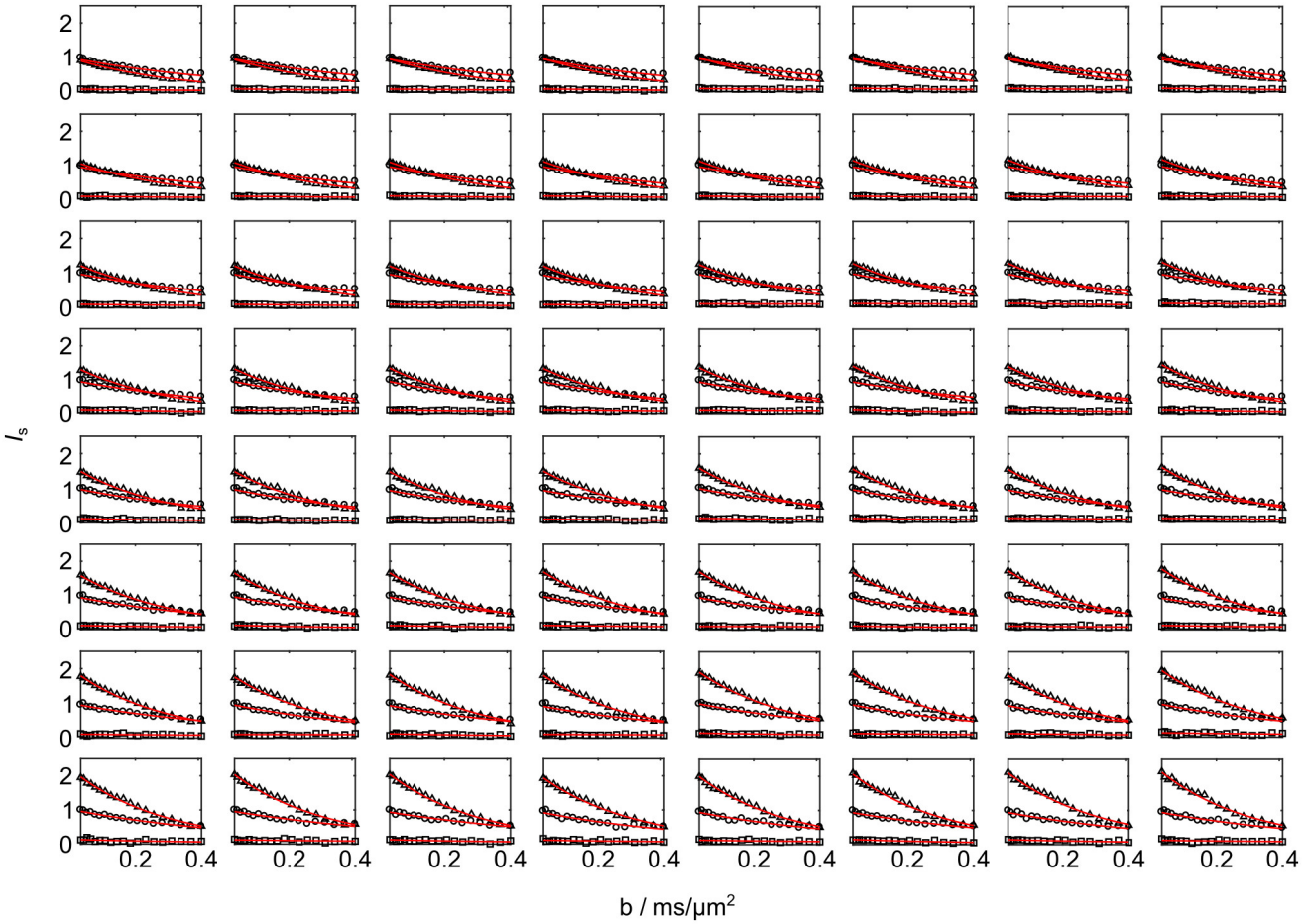

Figure S-8. Signals from 64 columns of hyperpolarized *D*-T_2 data after Fourier transform of each echo in the diffusion dimension, shown as a function of *b* and fitted with $I_s = I_0 \cdot exp(-b \cdot D)$ (red curve). $b = (\gamma G_{diff} \delta_{eff})^2 (\Delta - \delta_{eff}/3)$ ranged from 0.026 ms/μm^2 to 0.410 ms/ μm^2. Each panel corresponds to signal decay from a column of the corresponding data set. The column number increases from left to right starting from the top row. Within each panel, triangle, circle and square symbols stand for hyperpolarized *D*-T_2 data of pyruvate selection without cell suspension, pyruvate selection with cell suspension and lactate selection with cell suspension, respectively. The *D* values from the fit are averaged as mean *D* = (3.3±0.2) · 10^{-9} m^2/s, (1.8±0.1) · 10^{-9} m^2/s and (1.0±0.5) · 10^{-9} m^2/s for the three experiments, respectively. The errors are from the standard deviation of *D* values. In each panel, signals are normalized to the maximum signal amplitude of pyruvate selection with cell suspension experiment.

References:

(1) Holz, M.; Heil, S. R.; Sacco, A. Temperature-Dependent Self-Diffusion Coefficients of Water and Six Selected Molecular Liquids for Calibration in Accurate ^{1}H NMR PFG Measurements. *Phys. Chem. Chem. Phys.* **2000**, *2* (20), 4740–4742.

(2) Ahola, S.; Zhivonitko, V. V.; Mankinen, O.; Zhang, G.; Kantola, A. M.; Chen, H.-Y.; Hilty, C.; Koptyug, I. V.; Telkki, V.-V. Ultrafast Multidimensional Laplace NMR for a Rapid and Sensitive Chemical Analysis. *Nat. Commun.* **2015**, *6*, 8363.