ARTICLE

Sweet Optimization: Glucose-Vitrified Samples for Hyperpolarizing Glutamine in Biological Studies

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^{1}H background signal

Figure S1 represents the intensity of this background signal compared to the thermal equilibrium signal of 3M [1-13C]acetate in 100 µL D_2O/H_2O (90/10) with 40 mM TEMPOL and 2M glucose at 4.2 K.

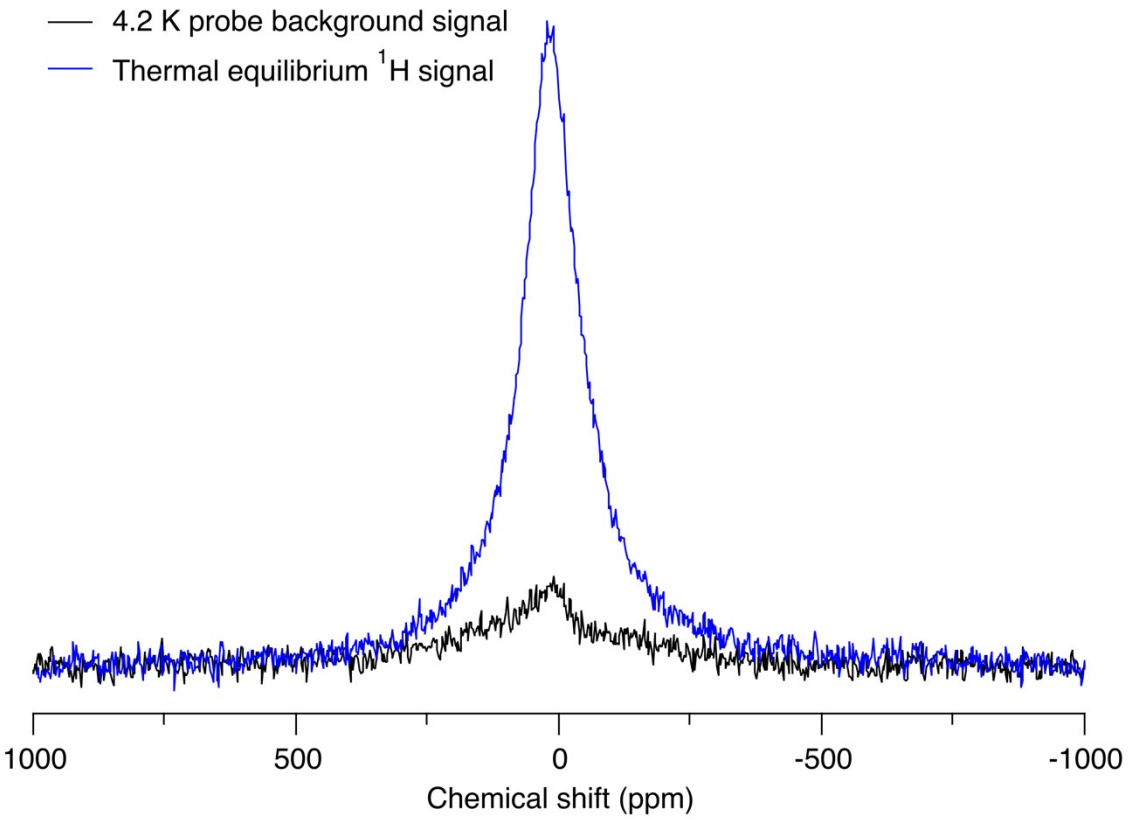

Figure S1. Comparison at 4.2 K of the thermal equilibrium of the probe's background ^{1}H signal (black) and the ^{1}H thermal equilibrium signal of 3M [1-13C]acetate in 100 µL D_2O/H_2O (90/10) with 40 mM TEMPOL and 2M glucose.

Estimation of the polarization buildup time constants

Figure S2 displays three ^{1}H polarization buildups at 1.2 K for three sample formulations: our 3 M and 0.25 M glucose formulations compared to a standard DNP sample using glycerol as a vitrifying agent.

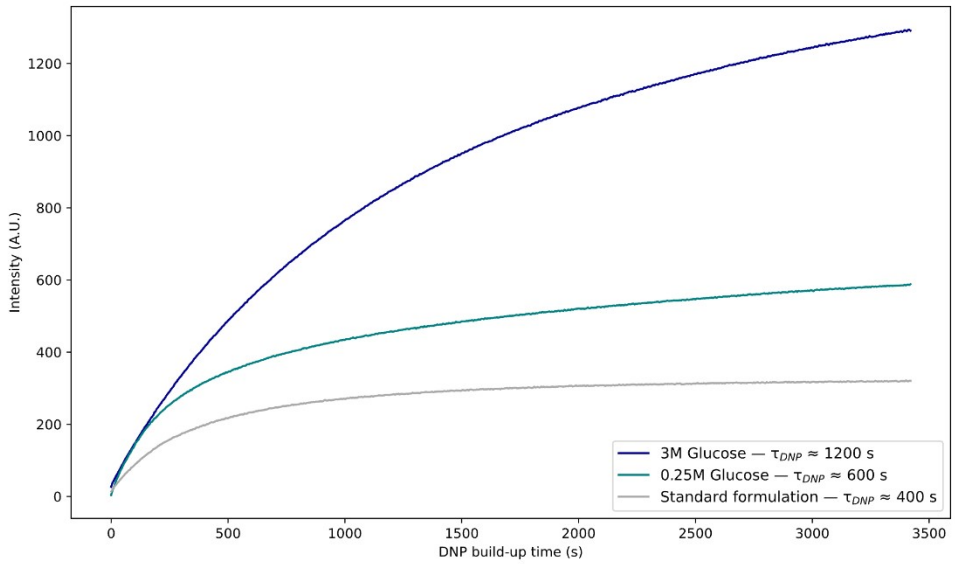

Figure S2. ^{1}H buildup profiles of formulations using 3 M glucose (in blue) and 0.25 M glucose (in green) and a standard DNP formulation using glycerol-d_8, D_2O and H_2O (60/30/10) (in grey). Experiments were performed with [1-^{13}C] sodium acetate at 3 M concentration using a 6.7 T polarizer at 1.2 K. One scan is measured every 5 s in these experiments.

The difference in intensity can be explained by the use of protonated glucose. However, the dynamics of these buildups are also significatively different with a buildup constant that can triple over the range of glucose concentrations studied in this work.

As illustrated below in Figure S3, it is possible to extract the buildup time constant τ_{DNP} of these experiments. For this purpose, we used a mono-exponential model, which may not be the best suited for all the experiments (which sometimes are better fitted using a bi-exponential or stretched exponential model) but provides nevertheless a good idea of the rate at which polarization builds up in each sample.
When polarizing using nitroxide radicals, this buildup time constant is highly important as the ^{1}H polarization buildup is used to polarize lower-gamma spins such as ^{13}C in our case using cross-polarization pulse sequences.

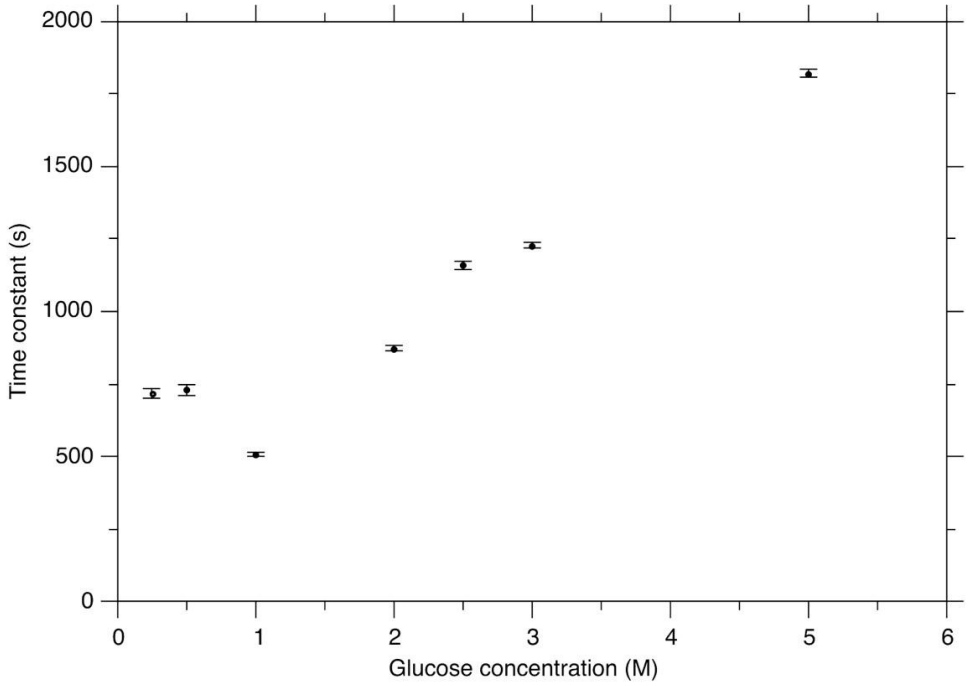

Figure S3. Mono-exponential time constants for ^{1}H nucleus obtained with [1-^{13}C]sodium acetate at a concentration of 3 M. Experiments were performed using a 6.7-T polarizer at 1.2 K, with glucose concentrations ranging from 0.25 to 5 M. The error bars correspond to the fitting error.

While there is no absolute limit, slow polarization buildups for ^{1}H are thus impractical for dissolution experiments and cross-polarization to ^{13}C. This can explain the best ^{13}C polarization in the series that we have measured for [1-^{13}C]sodium acetate prepared with 1 M glucose, as illustrated by its lower buildup time in Figure S3.

Polarization measurements

To determine the polarization levels, we calculate the ratio of the integrals of the signals with and without DNP and we correct them with respect to the differences in number of scans (NS) receiver gain (RG) and the angle of detection used for each experiment.

In solid state: The thermal equilibrium spectrum is measured at 4.2 K without using microwave irradiation. The DNP spectrum is measured at 1.2K using microwave irradiation (187.94 GHz, 120 mW before the frequency doubler). A ^{1}H background signal buildup for the probe was measured in order to subtract it from the measurements.

In liquid state: The DNP spectrum is the one with the better signal in the spectrometer and the thermal equilibrium spectrum is measured after the DNP experiment with the same sample.

The polarization can then be calculated using the following formula:

$$P(^{1}H)_{DNP} = \frac{I_{DNP}}{I_{TE}} \times \frac{RG_{TE}}{RG_{DNP}} \times \frac{NS_{TE}}{NS_{DNP}} \times P(^{1}H)_{TE} \quad \text{(S1)}$$

The polarization at thermal equilibrium is calculated using the formula derived from the Boltzmann statistics:

$$P = tanh(\frac{\gamma \hbar B_0}{2k_B T}) \quad \text{(S2)}$$

As mentioned in the main text, the probe's background signal (Figure S1) is subtracted from the sample's signal for polarization measurements.

Protonation rate in the DNP juice

Figure S4 presents the ^{1}H polarization values obtained as a function of the protonation rate in a [1-^{13}C]sodium acetate DNP sample.

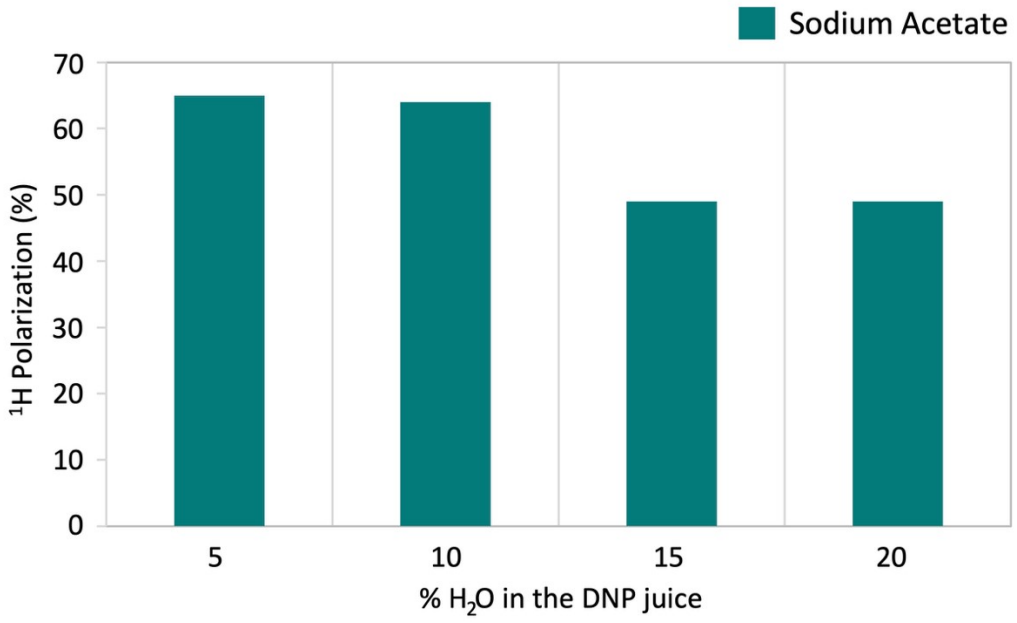

Figure S4. ^{1}H polarization of 3 M [1-^{13}C]sodium acetate as a function of H_2O content (5–20%) in the DNP juice. Experiments were performed using a 6.7 T polarizer at 1.2 K with 2.5 M of glucose.

Similar and efficient polarization values were observed for DNP samples containing 5% and 10% H_2O. Beyond 10% H_2O, the polarization efficiency began to decrease. Therefore, 10% H_2O appears to be an appropriate condition for subsequent experiments.

Dissolution experiments

Figures S5 shows the evolution of the [5-^{13}C] glutamine signal at 177.6 ppm after a blank dissolution without enzyme, the hyperpolarized signal being present for over two minutes.

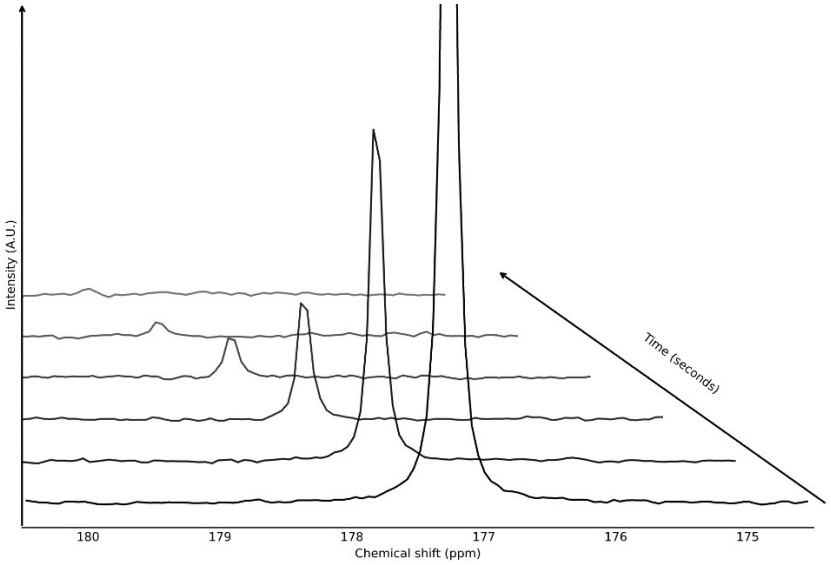

Figure S5. Intensity over time of a [5-^{13}C]glutamine signal (177.3 ppm) of a 5mL D2O dissolution observed for over 100 scans.

Fitting glutamine to glutamate enzymatic conversions

Figure S6 represents the fit obtained using equations 1 and 2 of the main text for one triplicate of the enzymatic conversion of glutamine to glutamate. This fit performed quite satisfactorily with a R^2 coefficient of 0.996 (with a value of 0.997 for both of the two other replicates). To further assess the pertinence of the model fitted to the data, we also extracted the residuals for glutamine and glutamate. They are shown in Figure S7.

With a maximum remaining below 5% for both datasets, they do confirm however that the early part of the experiment carries more differences between the data and the fit. As discussed in the main text, an explanation could be that the early part of the experiment is sampled too slowly compared to the relaxation and production / consumption dynamics of the system.

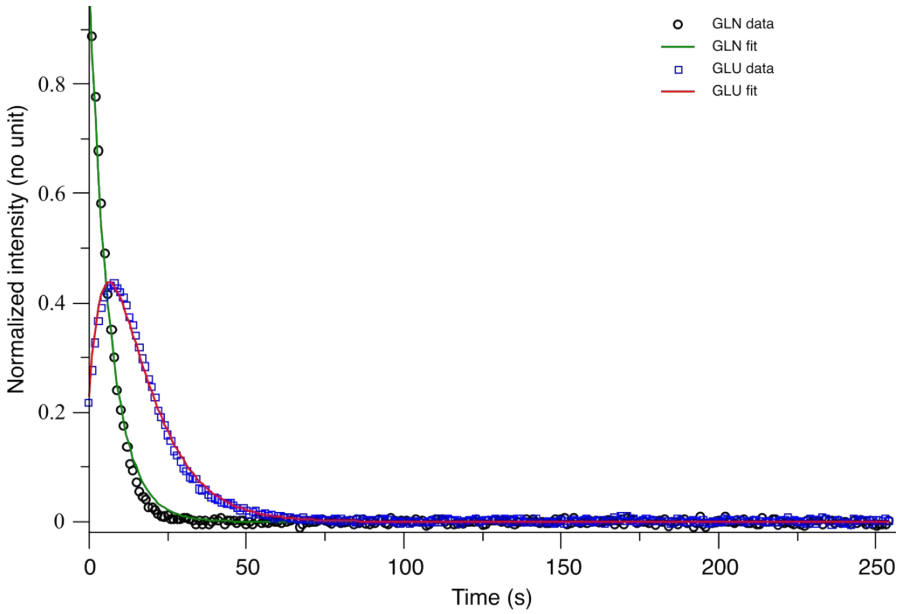

Figure S6. Fit of the glutamine and glutamate signal over time obtained using ODE fitting (symfit, python) for one of the triplicate experiments presented in the paper. The correlation coefficient for this fit is 0.995, and the residuals for each dataset is presented in Figure S7.

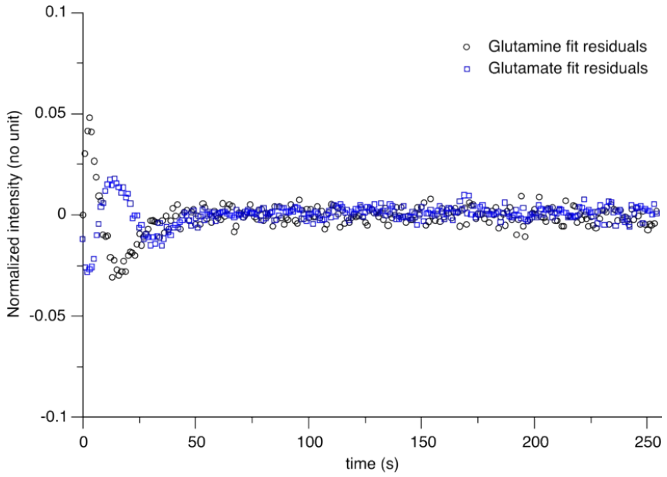

Figure S7. Residuals calculated for glutamine and glutamate over time using the fit presented in Figure S6. For each dataset, the maximal value of the residuals does not exceed 5% of the normalized experimental signal. The largest discrepancies observed are found in the earlies stages of the experiment where the dynamics of the system are slightly too fast with respect to the chosen sampling rate (1 Hz).

Extraction of the apparent conversion rates

We have tested the reproducibility of the reaction with glutaminase by monitoring glutamine conversion by glutaminase using dDNP in triplicate, at 100 U of enzyme and 400 MHz. The apparent kinetic rates k_{obs} determined through the analysis of the resulting data are reproduced from Table 1 of the article into Table S1 below.
To be able to compare these constants with our former works, a normalized rate, given in mM/L.s.U, was calculated from these k_{obs} using the enzyme activity (100 U) and the injected substrate concentration, which was quantified at thermal equilibrium after the dissolution experiments.

Table S1. Compilation of the fitted values for k_{obs} and the calculated values of k_{norm} for our triplicate dissolutions on glutaminase. For comparison, the values found in Dos Santos et al. are also given.

Source	k_{obs} (s^{-1})	[GLN] (mM)	Enzyme units	k_{norm} (mM/s.U.L)
Dissolution 1	0.12	5.3	100	6.20E-03
Dissolution 2	0.11	3.6	100	4.07E-03
Dissolution 3	0.17	5.5	100	9.35E-03
Dos Santos et al.	0.03	-	50	1.80E-03
Dos Santos et al.	0.06	-	50	3.70E-03
Dos Santos et al.	0.05	-	50	3.20E-03

We can notice that the values calculated for k_{norm} do differ from the publication from Dos Santos et al. Our main hypothesis is that the difference could be explained by the use of a different batch of glutaminase enzyme and by the temperature variation between the two studies (4 K), which may have affected the enzyme's efficiency. These factors, along with the difference in the number of units used, were the only changes between the two series of experiments. These values are sufficiently close, with only reasonable experimental variability, and remains within the same order of magnitude.

References

1 K. Dos Santos, G. Bertho, C. Caradeuc, V. Baud, A. Montagne, D. Abergel, N. Giraud and M. Baudin, Chemphyschem, 2023, 24, e202300151.