Diffusion MR of Hyperpolarized ^{13}C Molecules in Solution

Bertram L. Koelsch,[a,b] Kayvan R. Keshari,[a] Tom H. Peeters,[a] Peder E. Z. Larson,[a,b] David M. Wilson[a] and John Kurhanewicz[ab]*

[a] *Department of Radiology and Biomedical Imaging, University of California, San Francisco, USA. e-mail: john.kurhanewicz@ucsf.edu*
[b] *UC Berkeley – UCSF Graduate Program in Bioengineering, USA.*

Supporting Information

1. Data Acquisition. All MR studies were performed on a 14.1T Varian INOVA spectrometer (600 MHz ^{1}H/150 MHz ^{13}C) micro-imaging system (Agilent Technologies), equipped with a 10 mm broadband probe and 100 G/cm gradients. Probe temperature was controlled at 27 °C.

A pulsed gradient double spin echo sequence was used for all experiments (Fig. 1a). A 10° excitation pulse with a pair of adiabatic 180° refocusing pulses. This pulse sequence is particularly suited for quantitative hyperpolarized diffusion experiments because the adiabatic pulses are insensitive to transmitter-gain calibrations and the pair of 180° refocusing pulses realign the magnetization with the main magnetic field, thereby avoiding increased signal loss[1]. Since hyperpolarized signal is non-renewable, any small errors in a pulse sequence will propagate throughout an entire experiment and could complicate quantification. Diffusion measurements were interleaved with measurements used to determine the apparent T_1. Unless indicated otherwise, data were acquired every second (T_R = 1 s) for 150 seconds, with an echo time (T_E) of 50 ms. A crusher gradient (4 G/cm, 4 ms) was applied to saturate remaining transverse magnetization between every acquisition of the experiment.

Diffusion gradient pulses were positioned symmetrically around both 180° pulses with a gradient pulse duration (δ) of 5 ms and a gradient pulse separation (Δ) of 20 ms. By applying a range of gradient strengths (2 – 60 G/cm, in transverse orientation) spectra with different b-values (2 – 1500 s/mm^2) were obtained. To utilize the high SNR at the beginning of hyperpolarized experiments, b-values were arrayed from high to low. The b-value for two square gradient pairs[2] is defined by $b = 2\gamma^2 G^2 \delta^2 (\Delta - \delta/3)$, with γ the gyromagnetic ratio for ^{13}C. Spectra used to fit the apparent T_1 had a pair of crusher gradients (2 G/cm, 5 ms) around each of the adiabatic 180° pulses.

2. Hyperpolarization and Dissolution. Samples were polarized on a Hypersense (Oxford Instruments) and dissolved into 2 mL of a dissolution buffer, resulting in a final temperature of 27 °C. From this solution, 0.8 mL were rapidly transferred into an 8 mm susceptibility matched NMR tube (Shigemi Inc.), which was manually inserted into the bore of the spectrometer. Polarizations were measured by comparing the signal of the hyperpolarized sample with that of the thermally polarized sample. Convective effects were minimized by heating the spectrometer's bore to 27 °C (same as the sample temperature), by using a small sample volume that would reduce temperature gradients across the sample and by using diffusion gradients in the transverse plane (*e.g.*, G_x). Additionally, the comparison of hyperpolarized ^{13}C urea, measured in several seconds, with thermally polarized ^{13}C urea, measured over several minutes, confirms the ability to minimize convective effects in our diffusion measurements.

3. Thermal versus Hyperpolarized ^{13}C urea. Hyperpolarized ^{13}C urea diffusion coefficients were compared to those of ^{13}C urea at its thermal equilibrium polarization. Thermally polarized ^{13}C urea experiments were done on a 1 M solution, doped with 2 mM gadolinium to decrease the T_1 and thereby shorten the experiment time. These thermally polarized experiments used a 90° excitation pulse and a T_R of 10 s. The gradient strengths and thus b-values were the same as those used for the hyperpolarized experiments. The ^{13}C urea DNP sample was prepped according to a previously published protocol[3]. Hyperpolarized ^{13}C urea was dissolved in 2 mL deionized water and gave a final concentration of 16 mM. The measured diffusion coefficients for ^{13}C urea hyperpolarized and at its thermal equilibrium polarization were not statistically different; p-value = 0.20.

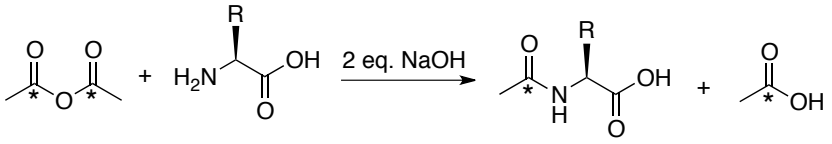
4. Simulation. We simulated the effects of both the apparent T_1 and the total diffusion measurement time on the accuracy of the calculated diffusion coefficient, using urea as our test case. With a previously published diffusion coefficient for urea,[4] adjusted to the temperature of our experiments, and the T_1 measured with a simple pulse-and-acquire experiment, we generated simulation diffusion data for hyperpolarized urea. Then, we modeled the correction of this simulation data using apparent T_1s that deviated from the true T_1 by ± 25% and with various total diffusion measurement times.

5. Hyperpolarized Diffusion of ^{13}C Pyruvate and ^{13}C Lactate. Both [1-^{13}C] pyruvate and [1-^{13}C] lactate were prepared according to previously published protocols.[3,5] The dissolution solution was a 50 mM phosphate buffer and the final concentration of these experiments was 11 mM.

6. Secondary Hyperpolarization with [1,1-^{13}C] Acetic Anhydride. Both protonated and perdeuterated [1,1-^{13}C] acetic anhydride were prepped according to a previously published protocol.[6] Signal enhancements after the chemical reaction were similar to those previously reported.[6] In separate experiments, acetic anhydride was reacted with glycine, triglycine or RGD (arginine-glycine-aspartic acid). The

dissolution solution for hyperpolarized [1,1-^{13}C] acetic anhydride contained 3 equivalents of the amino acid or peptide of interest and 2 equivalents of sodium hydroxide. This fast reaction resulted in hyperpolarized ^{13}C acetate and the acetylated version of the amino acid or peptide of interest. The absence of the [1,1-^{13}C] acetic anhydride in all spectra indicated that the reaction had gone to completion.

Scheme S1. The mechanism for secondary hyperpolarization of amino acids using hyperpolarized [1,1-^{13}C] acetic anhydride.



Diffusion coefficients of both [1-^{13}C] acetate and [1-^{13}C,d_3] acetate were measured at 26 mM while those for N-[acetyl-1-^{13}C] glycine and N-[acetyl-1-^{13}C,d_3] triglycine were done at a hyperpolarized concentration of 26 mM and a total concentration of 78 mM (since the amino acid/peptide was added at 3 times excess).

Diffusion coefficients for N-[acetyl-1-^{13}C,d_3] RGD were measured at a hyperpolarized concentration of 52 mM and a total concentration of 156 mM. For the N-[acetyl-1-^{13}C,d_3] RGD experiments, the T_R = 0.5 s, δ = 10 ms and b-values ranged from 150 – 5,400 s/mm^2. Additionally, the diffusion coefficient of N-[acetyl-1-^{13}C,d_3] RGD at its thermal equilibrium polarization was measured by using 15 averages per spectra at each b-value and required 12 h to complete. We calculated this diffusion coefficient to be 0.47×10^{-3} mm^2/s (n = 1).

7. NMR Data Analysis. All spectra were zero-filled to 8,000 points, line broadened 10 Hz and phase corrected (zero order). Integrated peak height and intensity were corrected for multiple excitations and the apparent T_1 was determined by fitting the exponential decay of the corrected signal. Subsequently, all diffusion data were also corrected for the apparent T_1 and for multiple excitations. From this, the diffusion coefficients (D) were determined by fitting the exponential $S/S_0 = \exp(-b \cdot D)$, where b are the b-values at each diffusion spectra. Six different diffusion weighted spectra were acquired for each dataset. S_0 is the hyperpolarized signal without diffusion weighting (b = 2 s/mm^2), but corrected both for the apparent T_1 and multiple excitations.

All data are presented as mean ± SD, n = 3. Statistical comparisons were made with Student's t-test and significance was considered to be at a p-value < 0.05.

8. Diffusion Weighted MRI. Diffusion weighted imaging was done with a pulsed gradient double spin echo and concentric echo planar imaging (EPI) readout. Hyperpolarized metabolites were excited with a 10° frequency specific Shinnar- Le Roux (SLR) pulse. During the 1 s T_R, ^{13}C urea, ^{13}C pyruvate and ^{13}C lactate were imaged with a field of view (FOV) of 25×25 mm (16×16 points). Diffusion coefficients maps were fit on a per-voxel basis in a region of interest (ROI) and are reported as ± SD. Otherwise, all pulse sequence parameters and data analysis methods were identical to those discussed above.

References

1. C. H. Cunningham, A. P. Chen, M. J. Albers, J. Kurhanewicz, R. E. Hurd, Y.-F. Yen, J. M. Pauly, S. J. Nelson, and D. B. Vigneron, *J Magn Reson*, 2007, **187**, 357–362.
2. K. Nicolay, K. P. J. Braun, R. A. de Graaf, R. M. Dijkhuizen, and M. J. Kruiskamp, *NMR in Biomedicine*, 2001, **14**, 94–111.
3. D. M. Wilson, K. R. Keshari, P. E. Z. Larson, A. P. Chen, S. Hu, M. Van Criekinge, R. Bok, S. J. Nelson, J. M. Macdonald, D. B. Vigneron, and J. Kurhanewicz, *J Magn Reson*, 2010, **205**, 141–147.
4. L. J. Gosting and D. F. Akeley, *J. Am. Chem. Soc.*, 1952, **74**, 2058–2060.
5. A. P. Chen, J. Kurhanewicz, R. Bok, D. Xu, D. Joun, V. Zhang, S. J. Nelson, R. E. Hurd, and D. B. Vigneron, *Magn Reson Imaging*, 2008, **26**, 721–726.
6. D. M. Wilson, R. E. Hurd, K. Keshari, M. Van Criekinge, A. P. Chen, S. J. Nelson, D. B. Vigneron, and J. Kurhanewicz, *P Natl Acad Sci Usa*, 2009, **106**, 5503–5507.