# Hyperpolarized Diffusion Weighted Carbon-13 MR

Bertram L Koelsch[1], Kayvan R Keshari[2], Renuka Sriram[2], Daniel B Vigneron[1,2], and John Kurhanewicz[1,2]
[1]*Graduate Program in Bioengineering, UC Berkeley - UCSF, San Francisco, CA, United States,* [2]*Radiology, UCSF, San Francisco, CA, United States*

**Introduction:** The development of dissolution dynamic nuclear polarization (DNP) has enabled MR measurement of real-time metabolism with hyperpolarized metabolites *(1, 2)*. These experiments are characterized by continuous signal loss, arising from the $T_1$ decay of the hyperpolarized signal but also from biological causes including perfusion, diffusion, cell membrane transport and enzymatic conversion of the hyperpolarized metabolites. Diffusion MR exploits differences in molecular motional properties to create contrast, weighted to local microstructural characteristics. Most diffusion weighted imaging and spectroscopy studies observe proton since carbon-13 is limited by low SNR, resulting from the nuclei's low gamma and its low natural abundance (1.1%). In this study, we take advantage of the signal enhancement achieved with dissolution DNP and molecular motion to acquire diffusion weighted spectra of hyperpolarized $^{13}$C urea. By comparing thermally polarized and hyperpolarized $^{13}$C urea diffusion we show the feasibility of using hyperpolarized diffusion weighted spectroscopy in future bioreactor and animal studies to quantify transport and enzyme kinetics.

**Experimental Methods:** All studies were done on a 14.1T (150MHz for $^{13}$C) wide-bore microimaging spectrometer equipped with 100 G/cm gradients and a 10mm broadband probe (Agilent Technologies). All measurements were done at 37$^{o}$C. Non-hyperpolarized/thermally polarized experiments were done with an aqueous 5M $^{13}$C urea solution. Hyperpolarized $^{13}$C urea was polarized in a HyperSense DNP polarizer (Oxford Instruments) and dissolved at 10mM in PBS *(3)*. A pulsed gradient spin echo sequence was used for diffusion experiments. The excitation angle (α) was 90$^{o}$ for thermally polarized samples and 5$^{o}$ for hyperpolarized samples. The $T_R$ was 300s for thermally polarized and 2s for hyperpolarized samples. Gradients applied in the x-direction were 10ms in duration (δ), 60ms in separation (Δ) and at strengths between 0 – 30G/cm. The apparent diffusion coefficient (ADC) of $^{13}$C urea was calculated by using the equation $\ln(S/S_o) = -\gamma^2 G^2 \delta^2 (\Delta - \delta/3) D = -bD$ *(4)*, where S is the measured signal at a specific gradient strength, $S_o$ is the signal at 5G/cm and the "b-value" represents the degree of diffusion weighting. Before determining the ACD, hyperpolarized spectra were corrected for flip angle and $T_1$ decay.

**Results and Discussion:** We implemented a pulsed gradient spin echo sequence to acquire quantitative hyperpolarized diffusion weighted spectra. We have adapted the sequence (**figure 1a**) for diffusion weighting hyperpolarized magnetization by using a small flip angle excitation pulse and a short $T_R$. The spin echo also refocuses $T_2$*, which is problematic at higher field strengths. A slab selective diffusion weighted 2D EPI image (**figure 1b**) of thermally polarized/non-hyperpolarized $^{13}$C urea illustrates the dependence of signal loss on the molecule's apparent diffusion coefficient (ADC) in a given environment. Rapid signal loss of $^{13}$C urea in water with increasing b-values shows that urea's ADC is 27 times greater in water than in glycerol. Signal loss is inherent to hyperpolarized experiments due to the inherent $T_1$ of the nuclei. Having measured hyperpolarized $^{13}$C urea's $T_1$ at 14.1T to be 43s in solution, we can reliably isolate hyperpolarized signal loss solely due to diffusion weighting (**figure 1c**). The ADC for thermally polarized $^{13}$C urea (15.5x10$^{-5}$ mm$^2$/s, $R^2$ = 0.993, n = 3) was measured in 1 hour with a 5M solution. The ADC of hyperpolarized $^{13}$C urea (17.3x10$^{-4}$mm$^2$/s, $R^2$ = 0.967, n = 3) was measured in seconds with a 10mM solution (**figure 1d**). It is noteworthy that determining the ADC of thermally polarized $^{13}$C urea at 10mM would require a multi-week experiment. The small difference between thermally and hyperpolarized ADC values can possibly be attributed to the effects of radiation damping and is under further investigation.

Prior studies have used diffusion weighting of protons to separate intra- and extracellular signals in an *in vitro* perfusion system *(5)*. This separation is possible because of the differences in the motional properties of molecules in these two environments. With a similar approach, we will use a bioreactor system developed in-house *(6)* in combination with diffusion weighting of hyperpolarized metabolites to separate intra- and extracellular signals. This experiment will allow for the simultaneous measurement of membrane transport and intracellular enzymatic conversion. This information is of particular interest, as it will elucidate the degree to which each of these factors contributes to detected hyperpolarized metabolic products. Further studies will extend the methodology developed here and in the proposed *in vitro* bioreactors studies to preclinical *in vivo* animal model studies.

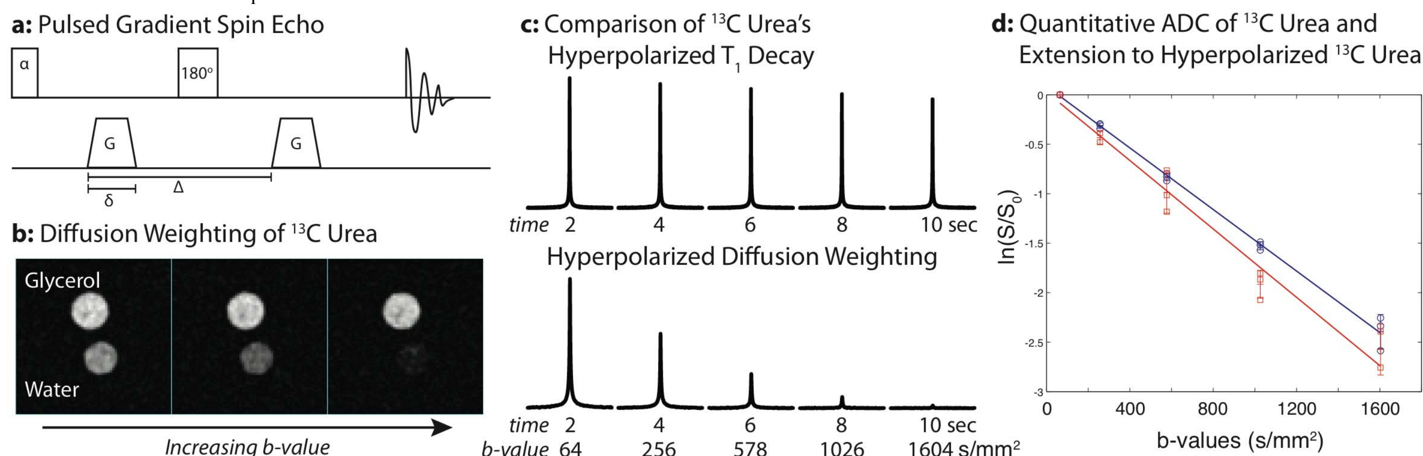


**Figure 1: (a)** The pulsed gradient spin echo sequence used. **(b)** A slab selective diffusion weighted EPI image demonstrating thermally polarized $^{13}$C urea's signal loss from increasing b-values. Solutions of different viscosities result in different apparent diffusion coefficients (ADC) for urea and accordingly stronger gradients suppress signal more quickly in water than in the more viscous glycerol. **(c)** Signal loss due to hyperpolarized $^{13}$C urea's $T_1$ is distinct from signal loss due to applying stronger gradients to achieve diffusion weighting. **(d)** The ADC for thermally polarized/non-hyperpolarized $^{13}$C urea (blue 15.5x10$^{-5}$mm$^2$/s) and hyperpolarized $^{13}$C urea (red 17.3x10$^{-4}$mm$^2$/s); ADC determined from the fit, n = 3, +/- SD.

**References:** 1. J. Ardenkjær-Larsen et al., *P Natl Acad Sci Usa* **100**, 10158 (2003). 2. K. Golman, R. in t Zandt, M. Thaning, *P Natl Acad Sci Usa* **103**, 11270–11275 (2006). 3. D. M. Wilson et al., *J Magn Reson* **205**, 141–147 (2010). 4. E. Stejskal, J. Tanner, *J. Chem. Phys.* **42**, 288 (1965).5. P. C. Van Zijl et al., *P Natl Acad Sci Usa* **88**, 3228–3232 (1991). 6. K. Keshari et al., *Magn. Reson. Med.* **63**, 322–329 (2010).

**Acknowledgements:** The authors thank Mark VanCriekinge, Subramaniam Sukumar and Deborah Hill for helpful discussions. Grant sponsor NIH P41 EB013598.