Dynamic Ultrafast 2D Exchange Spectroscopy (EXSY) of Hyperpolarized Substrates

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Target Audience: MR scientists interested in exchange spectroscopy or fast methodology to acquire 2D spectra; especially those interested in exchange and flux of hyperpolarized substrates.

Purpose: Magnetic resonance spectroscopy (MRS) of hyperpolarized substrates is a powerful tool for investigating tissue metabolism and kinetics *in vivo*[1]. In addition to detecting increased $K_{Pyr\rightarrow Lac}$ in tumors using MAD-STEAM, we recently showed that the backwards reaction, $K_{Lac\rightarrow Pyr}$, was significantly smaller in tumors compared to normal tissue with a transgenic model of prostate cancer[2], consistent with a decrease in LDHB expression. However, the $K_{Lac\rightarrow Pyr}$ as measured previously can be corrupted by alanine-to-pyruvate and hydrate-to-pyruvate conversion, warranting a method to separate these signals. Moreover, the directionality of reactions within the citric acid cycle has become an area of increased interest as reductive carboxylation has been shown to support tumor growth[3]. Here we present for the first time, a simple ultrafast method to acquire and reconstruct hyperpolarized 2D Exchange Spectroscopy (EXSY) dynamically.

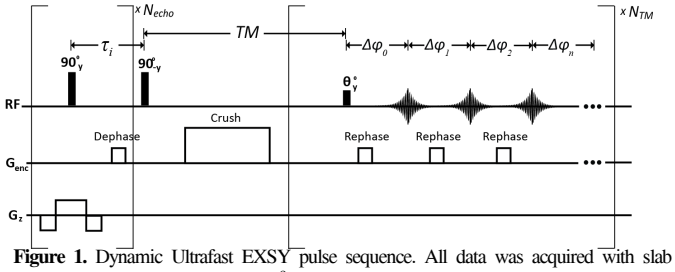

Figure 1. Dynamic Ultrafast EXSY pulse sequence. All data was acquired with slab selection in Δz=3mm, τ= 17.15, 20° flip, 1-2sec temporal resolution, 64 spectral points, 4006Hz bandwidth.

Theory: STEAM in the presence of metabolic conversion creates a phase shift dependent on the resonance frequency and echo time ($TE = 2\tau$), $\Delta\varphi = 2\pi f\tau$, which can be used to directly observe flux and exchange of a single reaction in real-time[4]. Concomitant signals at a single frequency can be resolved, with the acquisition of multiple echoes such that $\Delta\varphi$ varies significantly between the metabolites (Figure 2). If for at least one echo, $\Delta\varphi$ is between 0 and $\pm\pi$ and SNR is sufficient, the 2D spectra can be reconstructed accurately using the following equation:

$$S\{f_1,f_2\} = \begin{cases} \frac{Imag\{f_1,f_2\}}{\sin(2\pi(f_1-f_2)\tau)}, & f_1 \neq f_2 \\ Re\{f_1,f_2\} - \sum_i \frac{Imag\{f_1,f_i\}}{\tan(2\pi(f_1-f_i)\tau)}, & f_1 = f_2 \end{cases}$$

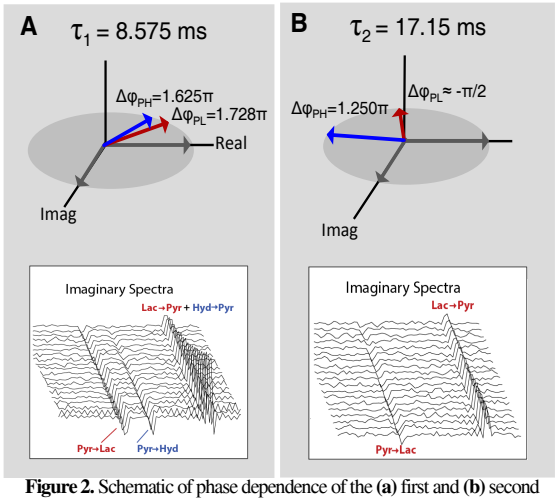

Figure 2. Schematic of phase dependence of the **(a)** first and **(b)** second echo and their corresponding raw dynamic imaginary spectra from UOK262 renal cell carcinoma cell-filled alginate microspheres (TR=1s). Alternate phase schemes could be used to maximize SNR in imaginary spectra (specifically to increase hydrate SNR). Up to an 83% increase in SNR can be recovered in the 2D spectra after reconstruction.

Methods: Studies were conducted on a 14.1T wide-bore microimaging spectrometer equipped with 100G/cm gradients and a 10mm broadband probe (Agilent Technologies). The sequence shown in Figure 1 was acquired with = 8.575*ms*, t_{phase} = 52 μs, G_{phase} = 5 *G/cm*, t_{crush} = 10 *ms*, G_{crush} = 15 *G/cm*, N_{echo} = 3, z = 3*mm*, *TM* = 1-2 *sec*, N_{TM} = 5-10. Cell studies were conducted in an MR-compatible bioreactor using UOK262 renal cell carcinoma cell-filled alginate microspheres with media at 37°C and 0.5mL/min[5], flow was turned off during acquisition. Noise was subtracted to remove cross peak artifacts and the T2 decay between echos was corrected.

Results & Discussion: Using MAD-STEAM single-voxel acquisition and reconstruction, real-time conversion and exchange can be directly observed for a pathway specified by the echo time (TE)[4]. Similarly, we utilize the phase accrual from the steam preparation pulses with a new acquisition and reconstruction method to observe multiple exchange pathways rapidly and simultaneously all within a single acquisition. The method was validated with Bloch simulations using SpinBench[7] and with hyperpolarized phantom experiments where the hydration of pyruvate was observed dynamically. We have also applied this technique to cell studies where both forward and backward exchange of pyruvate-lactate and pyruvate-hydrate was resolved and acquired dynamically (Figure 2,3). This is the first 2D EXSY experiment with hyperpolarized substrates.

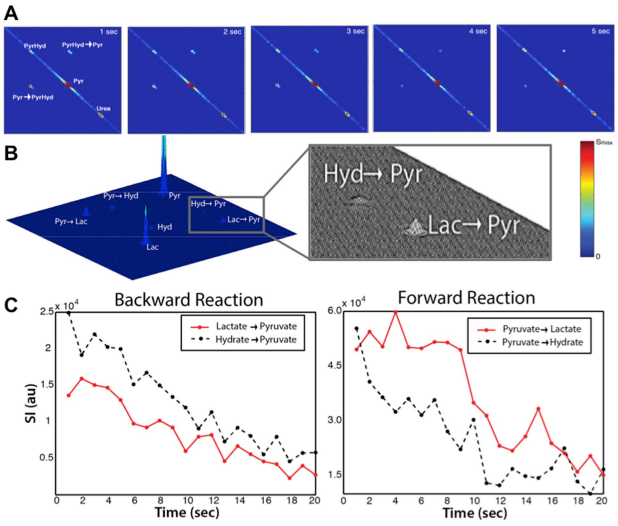

Figure 3. (a) 2D dynamic EXSY spectra of pyruvate hydration co-polarized with ^{13}C-Urea, **(b)** 2D sum spectra and **(c)** time course of pyruvate-lactate and pyruvate-hydrate forward and reverse reactions from MR-compatible bioreactor using UOK262 renal cell carcinoma cell-filled alginate microspheres.

Conclusion: In this work, we present a new ultrafast method for acquiring dynamic 2D Exchange Spectroscopy (EXSY) within a single acquisition using principles of MAD-STEAM. This technique reconstructs 2D EXSY spectra from 1D spectra based on phase accrual during the echo time. This approach has similarities to previous ultrafast 2D NMR techniques with the major difference being that simple STEAM encoding of discrete slices is used instead of frequency-sweep excitation pulses[6]. It requires only a single encoding step, and is thus ideal for dynamic imaging of many exchange pathways. Because it is a single-shot acquisition it is applicable to hyperpolarized substrates. This new approach could provide improved specificity to cancer metabolism in particular providing directionality of metabolic pathways. Outside of the field of oncology, the potential applications of this technique are broad including applications such as protein interactions and multistep chemical reactions[6].

References: [1] Kurhanewicz, et al. *Neoplasia.* 2011; 13(2): 81-97. [2] Swisher CL et al. *Magn Reson Med*, 2013; epub ahead of print. [3] Mullen et al. *Nature.* 2012; 481: 385-388. [4] Larson, et al. *J Magn Reson*. 2012;225:71-80. [5] K. Keshari et al. *Magn Reson Med*, 2010; 63: 322–329. [6] Mishkovsky and Lucio. *Chem Phys Chem,* 2008; 9(16): 2340–2348. [7] Overall and Pauly. ISMRM; 2007.

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