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Dynamic UltraFast 2D EXchange SpectroscopY (UF-EXSY) of hyperpolarized substrates

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ABSTRACT

In this work, we present a new ultrafast method for acquiring dynamic 2D EXchange SpectroscopY (EXSY) within a single acquisition. This technique reconstructs two-dimensional EXSY spectra from one-dimensional spectra based on the phase accrual during echo times. The Ultrafast-EXSY acquisition overcomes long acquisition times typically needed to acquire 2D NMR data by utilizing sparsity and phase dependence to dramatically undersample in the indirect time dimension. This allows for the acquisition of the 2D spectrum within a single shot. We have validated this method in simulations and hyperpolarized enzyme assay experiments separating the dehydration of pyruvate and lactate-to-pyruvate conversion. In a renal cell carcinoma cell (RCC) line, bidirectional exchange was observed. This new technique revealed decreased conversion of lactate-to-pyruvate with high expression of monocarboxylate transporter 4 (MCT4), known to correlate with aggressive cancer phenotypes. We also showed feasibility of this technique *in vivo* in a RCC model where bidirectional exchange was observed for pyruvate–lactate, pyruvate–alanine, and pyruvate–hydrate and were resolved in time. Broadly, the technique is well suited to investigate the dynamics of multiple exchange pathways and applicable to hyperpolarized substrates where chemical exchange has shown great promise across a range of disciplines.

1. Introduction

In the fields of chemistry and biology, multidimensional Nuclear Magnetic Resonance (NMR) acquisitions, which differentiate and correlate the resonances arising from individual sites onto multiple frequency axes, are commonly used to study structure, dynamics, reaction states, proteins, the chemical environment of molecules, or any other sample that contains nuclei possessing spin [1–3]. These experiments are intrinsically longer than their conventional one-dimensional (1D) counterparts. In general, 2D NMR techniques are limited by the inherent low sensitivity, resulting in acquisition times on the order of minutes to hours [1]. In carbon-13 NMR, this is particularly pronounced where less than 1% of carbon atoms possess the NMR detectable ^{13}C isotope. Moreover, SNR suffers from an intrinsically lower gyromagnetic ratio of the ^{13}C isotope.

Not surprisingly, there has been an increased interest in using nuclei in the 'hyperpolarized' state, whose spin population differences depart significantly from the $\approx 10^{-5}$ Boltzmann distribution. Dynamic Nuclear Polarization (DNP) yields over a 10,000-fold increase in SNR [2], which is far greater than what can be achieved by multiscan signal averaging. Hyperpolarization with its dramatic increase in sensitivity provides a unique opportunity to probe previously undetectable phenomena via NMR.

Signal detection of hyperpolarized substrates, however, is challenging due to nonrenewable longitudinal magnetization and short-lived signals. These challenges make conventional 2D NMR acquisition strategies incompatible with hyperpolarized substrates. Specifically, conventional acquisitions schemes for multidimensional NMR require an array of scans that are identical to one another aside from the serial incrementing of evolution delays. Given the non-renewable polarization and the shortened acquisition times due to signal decay by T_1, 2D NMR acquisitions with hyperpolarized substrates requires sequence modifications.

Shapiro and Frydman proposed a method for thermally polarized samples, where the serial indirect domain t_1 encoding of 2D NMR is replaced by a parallelized procedure allowing for different

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positions within a sample for inequivalent evolution times [4]. Then Frydman and Blazina extended this method to hyperpolarized substrates [1]. We propose a similar method utilizing their parallelized approach as the foundation for this work. The method presented here differs in several aspects: (1) it uses a symmetric slice selective excitation rather than a gradient acting in combination with a frequency-swept excitation for preparation and (2) it uses dephasing and rephasing gradients rather than an oscillating field gradient. The acquisition and reconstruction presented here relies on principles of phase accrual, which expands on our recently described 1D method Metabolic Activity Decomposition with Simulated Echo Acquisition Mode (MAD-STEAM) [5].

The key advancement presented in this work is its application to dynamic 2D EXchange SpectroscopY (EXSY) of hyperpolarized carbon-13 substrates, which we show can be used to detect bidirectional exchange. Exchange is particularly important, where both preclinical cell and animal studies of hyperpolarized substrates [6,9], as well as the first-in-man clinical trial [10], have focused heavily on the exchange of hyperpolarized metabolites as markers of disease.

Magnetic Resonance Spectroscopy of hyperpolarized substrates provides a new tool for investigating tissue metabolism and kinetics *in vivo* [11,12]. Previously experiments using MAD-STEAM showed that in addition to increased conversion of pyruvate-to-lactate in tumors, the less studied conversion of lactate-to-pyruvate was significantly smaller in tumors compared to normal tissue with a transgenic model of prostate cancer [13], consistent with a decreased LDH-B expression and increased monocarboxylate transporter 4 (MCT4) and LDH-A expression. However, the rate as measured can be corrupted by alanine-to-pyruvate and hydrate-to-pyruvate conversion, warranting a method to separate these signals to reveal the origin of this change.

Investigation of bidirectional flux and exchange has a number of applications to the metabolism field such as reductive carboxylation [14,15], lipogenesis and its regulation of citrate and α-ketoglutarate [16], glutamine addiction [17–20], gluconeogenesis, and the isoenzyme composition of LDH. Detection of these pathways has diagnostic and biomedical research potential. For instance, 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 [14]. However, the signal from hyperpolarized experiments reports only on the bulk spin-exchange and cannot differentiate concomitant spin-exchange. 2D NMR techniques for hyperpolarized substrates could be further used to probe directionality of metabolic pathways. Moreover, 2D NMR could provide improved specificity to cancer metabolism and shed light on exchange and flux of hyperpolarized substrates.

2. Theory

2.1. Acquisition

Conventional dynamic EXSY acquisition schemes necessitate renewable longitudinal magnetization not available in hyperpolarized substrates. Additionally, conventional EXSY acquisition schemes require many repetitions to obtain the entire indirect spectral dimension (Fig. 1a). The dynamic UF (ultra fast)-EXSY pulse sequence is rapid and does not require renewable longitudinal magnetization making it ideal for hyperpolarized substrates (Fig. 1b). Key features include the symmetric slice selection gradient played with the first 90° RF pulse, gradients blips, which rephase echoes sequentially, and a small flip angle for the final RF pulse, which allows for dynamic acquisition of 2D EXSY spectra. The data can be used to measure build up curves for multiple species that can be fit to an exchange model for extraction of kinetic rates of interconversion (Fig. 1c and d).

Since the method relies on stimulated echoes with gradient encoding, it is sensitive to motion and diffusion. For diffusion, high b-values can accelerate signal decay between TMs, shortening the measured T1 relaxation times ("effective T1" times[13]). In this work, we minimized the effect of diffusion by using short, low amplitude gradients, resulting in b-values <10 s/mm^2. Bulk motion would result in overall phase shift, however, a ^{13}C-Urea reference signal can be used to correct for the phase shift (Supplemental Fig. 1). Incoherent motion (e.g. turbulent flow) or non-rigid motion will result in additional unrecoverable signal losses. To prevent these losses in cell experiments, flow through the bioreactor was disabled during the signal acquisition.

This method also assumes spatial homogeneity within the voxel. To limit the effects of this assumption, we limited the number of echoes to the minimum requirement such that the inverse problem is not ill-posed. In the case of HP $^{13}C_1$ pyruvate only 2–3 echoes are required to resolve concomitant exchanging spins. This limits the size of the assumed homogenous region.

2.2. Reconstruction

Conventional EXSY reconstruction methods require many τ repetitions to reconstruct the 2D spectra. However, by choosing the τ repetitions wisely, only a few repetitions can be used to acquire an entire 2D sparse spectra. The UF-EXSY reconstruction, shown in Fig. 2, reconstructs the entire 2D spectra from only a few echoes (Fig. 2) with high spectral resolution in the indirect frequency dimension.

The reconstruction relies on the phase accrual, $\Delta\varphi$, of exchanging spins with a resonance frequency difference, Δf, at each echo time, τ, which has been used to directly observe flux and exchange of a single reaction in real-time [5]. For each frequency, f_i, with a signal greater than the noise threshold in the direct frequency direction the cross peaks are calculated using the following equation:

$$S(f_1,f_2)=\begin{cases}\frac{Imag\{S(f_1,f_2)\}}{\sin(2\pi(f_1-f_2)\tau)}, & f_1\neq f_2\\ Re\{S(f_1,f_2)\}-\sum_i\frac{Imag\{S(f_1,f_2)\}}{\tan(2\pi(f_1-f_2)\tau)}, & f_1=f_2\end{cases} \quad (1)$$

By using the real and imaginary spectra, the 2D spectra can be reconstructed from a single echo utilizing the phase accrual, $\Delta\varphi$, between all other frequencies with a signal greater than the noise threshold. However, multiple echo times need to be used to correct for concomitant exchange pathways at a single resonance such that $\Delta\varphi$ varies between the exchange pathways. Additionally it is required that for at least one echo the phase accrual does not equal zero ($\Delta\varphi\neq 0$). With these criteria fulfilled, the problem is no longer an ill-posed inverse problem. As the number of echoes increases, the accuracy will increase. To ensure accuracy at least one unique echo is required for each concomitant spin exchange. For instance in the renal cell carcinoma model, UOK262, there are three possible concomitant spin exchanges at pyruvate's resonance, namely lactate-to-pyruvate, hydrate-to-pyruvate, and alanine-to-pyruvate. Because alanine SNR is below the noise threshold, we only need two echoes to accurately reconstruct the data (Fig. 2).

There will be a small loss in SNR at each repetition due to parsing of the signal. Fortunately, much of the original SNR can be recovered. Where the SNR of a cross peak is a function of τ_i and is defined by

$$SNR(\Delta f)=\frac{S}{n\sigma}\left(\frac{1}{\sin(\Delta\varphi(\tau_1))}+\frac{1}{\sin(\Delta\varphi(\tau_2))}+\cdots\frac{1}{\sin(\Delta\varphi(\tau_n))}\right) \quad (2)$$