Hybrid frequency encoding/water relaxation method for detecting exchangeable solute protons with increased sensitivity and specificity

Nirbhay N Yadav[1,2], Jiadi Xu[1,2], Xiang Xu[1,2], Michael T McMahon[1,2], and Peter C M van Zijl[1,2]

[1]*Radiology, The Johns Hopkins University, Baltimore, MD, United States,* [2]*FM Kirby Research Center, Kennedy Krieger Institute, Baltimore, MD, United States*

TARGET AUDIENCE: Researchers and clinicians interested in detecting mM concentration metabolites in vitro or in vivo.

PURPOSE: To show that water relaxation in the presence of metabolites with exchangeable protons is affected by the exchange regime for the metabolite protons and that the specific relaxation contributions can be measured using frequency labeling of the metabolite protons and subsequent detection of the transfer of this label to the water signal.

INTRODUCTION: Chemical exchange between water and labile protons is a fundamental process in molecular dynamics and its measurement is critical in the characterization of many phenomena including molecular structure and kinetics. The magnetic resonance method of choice for reporting on chemical exchange typically depends on the relationship between exchange rate (k_{sw}) of the exchangeable proton to water and the difference in resonance frequencies between the exchanging pools ($\Delta\omega$). Recently, Chemical Exchange Saturation Transfer (CEST)[1] has shown great promise for detecting low-concentration solutes with exchangeable protons. The FLEX method (Fig. 1A)[2] possesses capabilities similar to CEST, but, unlike CEST, which detects a reduction in water signal intensity, FLEX modulates the water signal intensity by encoding the chemical shift of each proton pool. For CEST and FLEX experiments, the exchanging solute protons need to be chemically distinct from the bulk water pool (i.e., in the slow-intermediate exchange regime; $k_{sw} \leq \Delta\omega$). Above this limit, the solute proton peak disappears from the NMR spectrum and is only revealed through broadening of the strong water signal. One method for measuring protons in this exchange regime (intermediate-fast) is T_2 relaxation dispersion (RD).[3] RD quantifies the water line-broadening by measuring the water signal intensity after a CPMG pulse train module of fixed total length but as a function of echo spacing. Remarkably, here we report that the FLEX evolution period is also sensitive to chemical exchange based water line broadening and thus can be used to quantify the contribution of the chemical exchange of protons in the intermediate-to-fast exchange regime to the water relaxation. In addition, the information that is available from the modulation of the water signal intensity can be used to identify the source of the proton. This combines the increased sensitivity of exchange transfer with specific assignment of the proton origin. Here, we studied three common brain metabolites and used the FLEX method to extract both chemical shift and exchange rate information for the exchangeable protons of each compound and show how they affect water relaxation.

METHODS: Creatine (Cr), myo-inositol (MI), and L-Glutamate (Glu) were dissolved in PBS at concentrations of 10 mM, 7.5 mM, and 12.5 mM respectively. Solutions were titrated to pH 7.3 and then transferred to 5 mm NMR tubes. FLEX MRI experiments were conducted at room temperature on a 11.7 T Bruker Biospec system equipped with a 40 mm transceiver coil. The FLEX sequence (Fig. 1A) consisted of labeling pulses applied on the water resonance [4] (duration: 30 μs, amplitude: 196 μT) and the time domain signal was generated by varying t_{evol} from 0.0 to 4.0 ms in steps of 0.05 ms (dwell time). The total preparation period consisted of 750 LTMs (each 7 ms in duration) and was followed by a single-shot fast spin-echo readout.

RESULTS AND DISCUSSION: Fig. 1B shows Bloch simulations for 50 mM protons at a frequency offset of 3 ppm ppm from water and multiple exchange rates using the experimental pulse sequence parameters. At very slow rate, frequency labeling is complete but negligible signal is transferred and no frequency modulation or relaxation enhancement is measured. When the rate increases, both signal modulation and relaxation enhancement (faster decay) become visible. In the intermediate-fast exchange regime mainly relaxation enhancement occurs, and when exchange is really fast frequency labeling does not occur before exchange and the relaxation enhancement disappears again giving a water decay rate similar to slow exchange. Fig 1C shows the FLEX time-domain signals acquired (circles) from Cr, MI, and Glu. Fitting the Bloch equations to the experimental data (solid lines) yields a peak at 2.0 ppm with k_{sw} = 240 s^{-1} for Cr, 1.1 ppm with k_{sw} = 1381 s^{-1} for MI, and 2.6 ppm with k_{sw} = 6500 s^{-1} for Glu. At 11.7 T, these exchange rates represent protons in the slow (Cr), intermediate (MI), and intermediate-fast (Glu) exchange regimes. It would be possible to detect the chemical exchange of these compounds by simply using a RD sequence however the RD signal is composed of a weighted sum of different protons groups and thus cannot easily separate multiple contributing components. FLEX has the advantage of an additional modulating signal component that can distinguish and separate out different groups of protons. This is particularly important for solutions containing multiple components and in vivo.

CONCLUSION: Here we show that the FLEX signal decay rate is sensitive to slow-intermediate-fast exchanging protons and, when the exchange is in the appropriate exchange regime, it can provide specific chemical shift information on exchanging protons. This method may be useful when detecting multiple groups of protons with a large range of exchange rates, for instance metabolic products.

REFERENCES: [1] Ward et al., J Magn Reson 2000; 143: 79-87. [2] Friedman et al., JACS 2010; 132: 1813-1815. [3] Xu et al., ISMRM 2013; 4240. [4] Yadav et al. Magn Reson Med 2012; 68:1048-55;

FUNDING: R01EB015032 & EB015031, P50CA103175, P41015909.

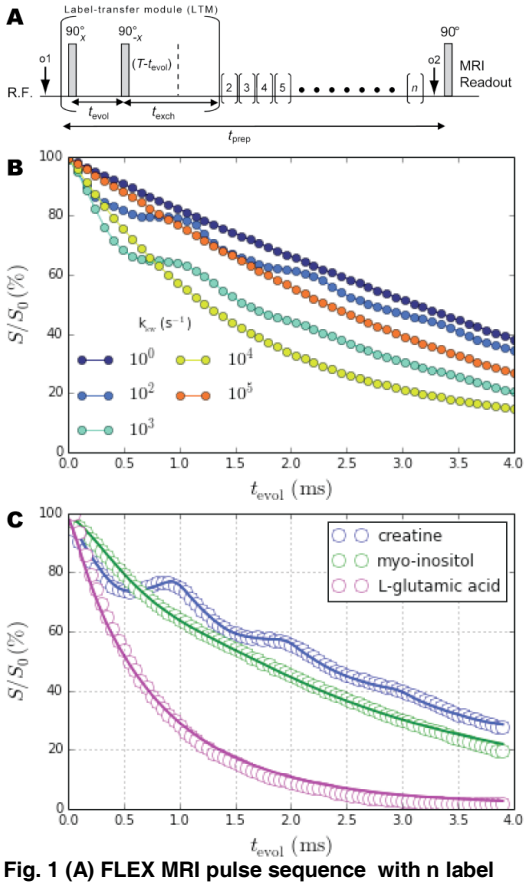

Fig. 1 (A) FLEX MRI pulse sequence with n label transfer modules (LTMs) (B) Bloch simulations showing the effects of chemical exchange rate on the FLEX time-domain signal. (C) FLEX experimental data (circles) and fit (lines) from phantoms of some common brain metabolites.