T2 Relaxation Dispersion Technique to Detect Intermediate and Fast Exchanging Protons in Metabolites and Proteins

Jiadi Xu[1,2], Nirbhay Yadav[1,2], Kannie Chan[1,2], Amnon Bar-Shir[2], Qin Qin[1,2], Guanshu Liu[1,2], Michael McMahon[1,2], and Peter van Zijl[1,2]

[1]F. M. Kirby Center, Kennedy Krieger Institute, Baltimore, MD, United States, [2]Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University, Baltimore, MD, United States

Target audience: Scientists interested in mapping metabolite and/or protein distribution or pH. Investigators interested in measuring the chemical shifts and exchange rates of diaCEST/paraCEST contrast agents with intermediate and fast exchange rates.

Purpose: Chemical Exchange Saturation Transfer (CEST) is a popular technique for generating MRI contrast based on the presence of exchangeable protons in low concentration metabolites and proteins in vivo.[1] CEST of endogenous or diamagnetic species is commonly used for protons in the slow to intermediate exchange regime, i.e., $k<=2\pi\Delta\omega$, where k is the exchange rate, and $\Delta\omega$ is the chemical shift difference with water. While new methods are being developed that extend the detection limit to more rapidly exchanging protons (i.e., on-resonance FLEX[2] and T1ρ techniques), it is important to develop simple methods to study the high intermediate to fast exchange limit, i.e. $k>2\pi\Delta\omega$, where the exchangeable proton peaks merge with water peak. Exchangeable protons such as NH_2 and OH groups that are common in metabolites and proteins fall into this category. We propose to use T_2 relaxation dispersion (RD) to detect protons in this intermediate-fast exchange regime. These fast exchanging solute protons, which are invisible in the NMR spectrum, are revealed as line-broadening of the strong water signal, especially at higher magnetic field where $\Delta\omega$ increases in frequency units[3]. Quantifying such water line-broadening can be used to extract out the exchange rates and chemical shifts of the exchangeable protons. The T_2 RD experiment employs a Carr-Purcell-Meiboom-Gill (CPMG) pulse train module of fixed total length, followed by a flip-back -90 degree pulse, that is applied before the image readout as shown in Fig. A. The signal intensity $I(\nu_{CPMG})$ of the water is monitored as a function of the repetition rate of the refocusing pulses in this module, defined as $\nu_{CPMG}=1/(2t_{cp})$, where t_{cp} is the separation between the pulses.[4] This echo spacing is varied by changing the number of 180-degree pulses during the total echo time. (Fig. A).

Methods: N-Acetylaspartic acid (NAA), Creatine (Cr), Lactate (Lac), L-Glutamic Acid (Glu), KH_2PO_4, Choline (Cho) and myo-inositol (mI) were dissolved in phosphate buffered saline to form solutions with concentrations of 12.5 mM, 10 mM, 5 mM, 12.5 mM, 50 mM, 3 mM and 7.5 mM, respectively to represent the typical metabolites in brain. Solutions were titrated to pH 7.3, and then transferred to 5 mm NMR tubes. MRI experiments were conducted on a horizontal 11.7T Biospec system (Bruker BioSpin). Transmit and receive were achieved using a 23 mm quadrature volume resonator and all experiments were performed at room temperature. Images for RD experiments and CEST experiments were acquired using a RARE readout with TR=20 s, TE=10 ms, NA=1, slice thickness 1 mm, and 64×64 matrix were used (FOV 2.0×2.0 cm^2). In the CPMG preparation module, the total echo time, t_{echo}, was set to 40 ms. The 90 degree and 180 degree pulses were 0.03 ms and 0.1 ms respectively. The number of refocusing pulses in the CPMG module was varied from 4 (ν_{CPMG} = 50 Hz) to 224 (ν_{CPMG} = 2.8 kHz). The $R_{2,eff} = 1/T_{2,eff}$ values at repetition rate ν_{CPMG} =50 Hz were measured using same sequence by increasing the total echo time and keeping the echo spacing 10 ms, while the $R_{2,eff}$ values at other ν_{CPMG} frequencies are calculated using the following relationship:[4]

$$R_{2,eff} = R_2(\nu_{CPMG}) = R_2(50) - \ln(I_{\nu_{CPMG}} / I_{50}) / t_{echo}$$

The CEST Z-spectrum was recorded using a 2 s continuous wave saturation with power level 1 μT before imaging.

Results and Discussion: The CEST Z-spectra and T_2 RD curves of Glu, Cr and mI are plotted in Figs. A and B, resp. The exchangeable protons in these molecules are amines (NH_2) in Glu, the guanidyl protons ($NHNH_2$) in Cr and OH groups in mI. At 11.7T and pH = 7.3 in PBS, these represent slow (Cr), intermediate (mI) and intermediate-fast exchanging protons (Glu), respectively. It can be seen from the CEST Z-spectrum that the exchangeable proton peaks overlap with the water peak in the intermediate and fast exchange situation but that their effects are still detectable by CEST (contrary to NMR). However, it is difficult to quantify exchange rates in the fast regime when performing CEST experiments. Conversely, the chemical shifts, exchange rates, and T_2 values of the exchangeable protons can be obtained from the fitted RD curves (values are listed in table) using the Bloch equations.[4] In Fig. A, arrows indicate the chemical shifts of the exchangeable solute peaks. As expected, no exchangeable proton peaks are observed for KH_2PO_4, while the exchangeable protons (OH groups) in Lac, Cho and NAA are also hard to be observed using both CEST and T_2 RD techniques due to the small chemical shifts.

Metabolite	Chemical Shifts (ppm)	Exchange Rate (kHz)	T_2 (ms)
Cr	1.5	0.5	36
mI	0.82	1.84	33
Glu	2.67	6.23	2.2

Conclusion: T_2 RD is a sensitive technique for detecting low concentration metabolites with intermediate or fast exchanging protons. As opposed to CEST, this technique has the disadvantage of measuring the total pool of exchangeable protons in the system, but this may have advantages when needing high SNR for instance for studying pH. Other potential applications may be to detect the presence of exchange-based contrast agents[5] or for studying physiological changes in a single metabolite during a task.

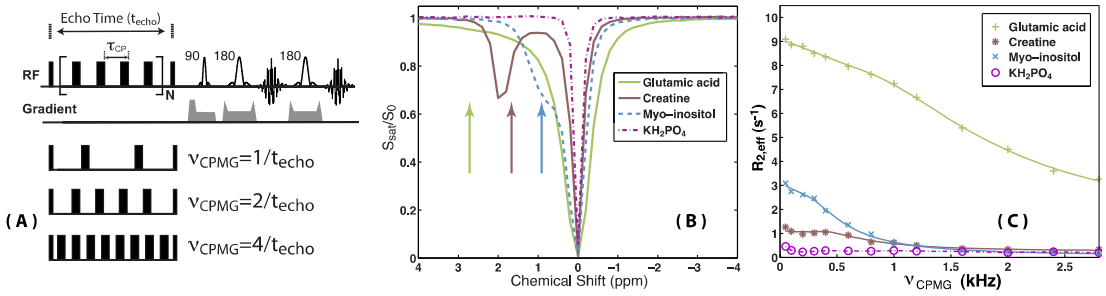

Figure 1. (A) The pulse sequence for the T2 RD experiments. (B) CEST Z-spectra and (C) the T_2 RD curves of Glu, Cr, mI and KH_2PO_4, respectively.

References:

1. van Zijl & Yadav. MRM 2011;65: 927. 2. Yadav et al. MRM 2012;68:1048. 3. Yadav et al. this meeting. 4. Korzhnev et al. Nature 430:586. 5. Soesbe et al. MRM 2011;66:1697.

Funding Support:
NIH grants R01EB15031, R01EB015032, P50CA103175, 1S10RR028955 and P41EB015909