

Detection of paraCEST Agents with Reduced MT Interference Using Frequency Labeled Exchange Transfer (FLEX)

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Introduction: Currently, there is an increasing effort to design paramagnetic chemical exchange saturation transfer (paraCEST) agents for biological target identification by MRI (1,2). The main benefit of paraCEST agents over their diamagnetic counterparts is that they can be engineered to have large chemical shift differences ($\Delta\omega > 50$ ppm) between the exchanging pools. Consequently, saturation pulses can be applied well away from the water resonance, thus avoiding off-resonance saturation of bulk water. In addition, faster exchanging compounds can be used while still adhering to the slow exchange limit ($k_{ex} < \Delta\omega$) necessary for CEST. While this allows large exchange transfer effects to be measured, such rapid exchange rates (k of 10^4 - 10^5 s $^{-1}$) require fast RF saturation labeling, which needs to be achieved by increasing the B_1 level of the radiofrequency (RF) irradiation. Unfortunately, this comes with an increase in conventional magnetization transfer (MT) effects in biological tissues, which, despite the large $\Delta\omega$, can still complicate the analysis in paraCEST studies. Recently, a novel sequence dubbed frequency-labeled exchange transfer (FLEX) was proposed, which detects exchanging protons using frequency transfer instead of saturation transfer (3). Unlike CEST, which is based on a drop in the water signal intensity due to saturation, FLEX detects the modulation of water signal as a function of evolution time (t_{evo}) which can be described by a free induction decay (FID). This signal modulation in the time domain provides an opportunity to remove rapidly decaying components with short T_2^* (e.g. MT effects). The aims of this study were to evaluate the FLEX technique for rapidly exchanging paraCEST agents ($k_{ex} > 10000$ s $^{-1}$) and its ability to suppress MT interference in an agarose phantom.

Material & Methods: A 10 mM solution of Eu³⁺-DOTA-(gly)₄ paraCEST agent (4) was mixed with 4% agarose (to simulate semi-solid MT effects). Experimental data was acquired at 37°C on a Varian Inova NMR spectrometer operating at a ¹H resonance frequency of 600 MHz. The FLEX sequence used in this study is described as Fig. 1. Gradients are not shown, but these need to be applied during exchange and readout to avoid radiation damping in phantoms. The FLEX FID signal was acquired by varying t_{evo} from 5 μ s to 245 μ s using time intervals of 3 μ s (dwell time). The offset frequency (ω_1) = 51949 Hz (86.5 ppm) was calculated to avoid water excitation. The other parameters for FLEX data acquisition were: t_{exch} = 300 μ s and number of label transfer modules (LTMs, see Fig. 1) = 1, 100, 300, 500, 700, and 1000. Signal differences with respect to the average water signal after decay were normalized with respect to the water signal with negligible evolution.

Results & Discussions: In Fig. 2, a conventional CEST spectrum is displayed (black line). It can be seen that a strong MT profile significantly obscures the paraCEST peak. Asymmetry analysis and observing the deviation from a straight line reveal a peak at \sim 50 ppm (Fig. 2, blue and red lines, respectively). The CEST effect obtained by asymmetry analysis seems too large, probably due to additional asymmetry in the agarose CEST spectrum. The deviation of the CEST spectra from a straight line gives a CEST effect of \sim 4%. In Fig. 3, a representative FLEX FID (500 LTM case) is shown (inset), indicating a rapid signal drop in the first 20-30 μ s, followed by a more gradual decay. When analyzing the full FID, a broad resonance is found that obscures all signals. However, when filtering the rapidly decaying broad component (in red, short T_2^*), the resulting spectrum shows the correct frequency for the paraCEST agent at about 50 ppm. No asymmetry analysis is needed for this data processing. The peak corresponding to the location of the Eu³⁺-DOTA-(gly)₄ paraCEST agent is much larger than the water signal due to the limited excitation of bulk water when using an ω_1 of 86.5 ppm. The FLEX signal is amplified by increasing the number of LTMs (Fig. 4) because it allows multiple opportunities to transfer labeled protons to the water. The exchange rate was obtained using time domain analysis (3), giving $k_{ex} \sim 24000$ s $^{-1}$. By observing the FLEX effect as a function of number of LTMs (Fig. 4) and using the FLEX theory (3), we determined the concentration of the paraCEST agent to be 12.0 mM (corresponding reasonably well to the known concentration of prepared paraCEST agent (10 mM)). In addition, this fit provided $T_{1w} = 545$ ms.

Conclusion: The first results from FLEX experiments on a paraCEST agent in agarose at physiologic temperature were presented. The FLEX spectra contained a peak corresponding to the location of the paraCEST agent in CEST experiments, but with negligible MT interference. These findings demonstrate potential for *in vivo* imaging with reduced dosage of paraCEST agent and without need for asymmetry analysis to remove MT contributions.

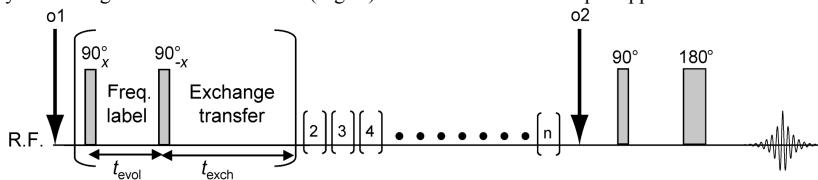


Fig. 1. Schematic diagram of the FLEX pulse sequence used in this study. FLEX consists of a series of n label-transfer modules (LTMs) in which exchangeable solute protons are frequency labeled and transferred to water. Within each module, a pair of selective $90^\circ_x/90^\circ_{-x}$ RF pulses are applied, in between which chemical shift evolution of the exchangeable protons occurs during the period, t_{evo} for frequency labeling. After storage of the frequency information in the form of longitudinal magnetization by the 90°_x pulse, a waiting period, t_{exch} , is applied to allow exchange transfer to the solvent. Signal amplification occurs because fresh z -magnetization is present for solute protons at the start of each LTM.

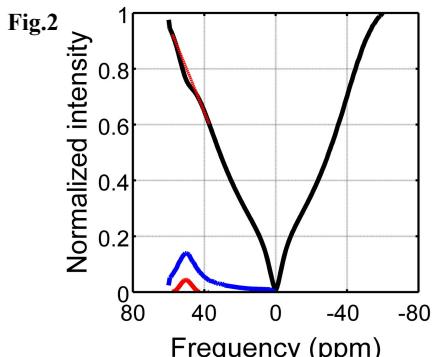
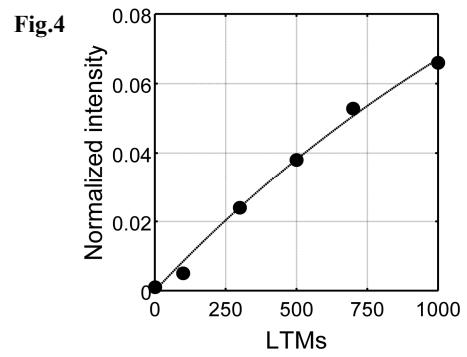
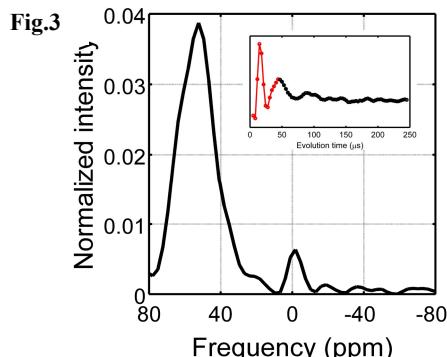


Fig. 2 CEST spectra and asymmetric analysis of a Eu³⁺-DOTA-(gly)₄ paraCEST agent mixed with agarose. CEST spectra was measured by applying a presaturation pulse with $B_1 = 10$ μ T and saturation time of 4 s. The frequency of the saturation pulse was varied between ± 60 ppm in the steps of 0.67 ppm. **Fig. 3** FLEX spectrum for 500 LTMs obtained by Fourier transform of the water signal modulation as a function of t_{evo} (inset) which contain the multiple frequencies. The early part of time signal (red line) associated with rapidly decaying component of MT effect was discarded. The signal intensity of FLEX spectra was normalized to the average of the water signal in the time domain for LTM=1. **Fig. 4** Concentration of labeled protons generated by FLEX as a function of the number of applied LTM's. Black line is best fit of the data to the FLEX theory.



Reference: 1. S. Zhang, et al., JACS, 123: 1517-1518, 2001. 2. S. Aime et al., JACS, 124: 9364-9365. 3. J. I. Friedman, et al., JACS, 132: 1813-1815, 2010. 4. S. Viswanathan, et al., Angew. Chem. Int. Ed., 48:9330-9333. This study supported by NIH/NCRR grant 5P41RR002584.