Detection of Chemical Exchange Saturation Transfer (CEST) Contrast Using Frequency Transfer

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Introduction: Chemical Exchange Saturation Transfer (CEST) MRI employs contrast based on transfer of saturation from exchangeable protons of low-concentration solutes to water protons (1-4). Currently, CEST agents are detected using radiofrequency (RF) based saturation spectra (z-spectra) followed by magnetization transfer asymmetry analysis around the water frequency. We report a new approach that, instead of saturation transfer, employs a series of so-called label-transfer modules (LTMs) achieving frequency labeling and consecutive exchange transfer of labeled protons to water. This Frequency-Labeled-EXchange (FLEX) method provides a sensitivity enhancement similar to CEST, but adds the possibility of exchange rate filtering and simultaneous study of multiple exchangeable protons.

Methods: The pulse sequence (Fig. 1) consists of n LTMs in which exchangeable solute protons "s" are frequency labeled and transferred to water, "w". Within each module, we employ binomial frequency labeling using a pair of selective $90_x/90_{-x}$ RF pulses, in between which chemical shift evolution of the exchangeable protons occurs during the period, t_{evol}. 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, where labeled protons are stored longer-term as water protons. Signal amplification occurs because fresh z-magnetization is present for solute protons at the start of each LTM, allowing multiple opportunities to transfer labeled protons to the solvent when applying multiple (n) modules during the preparation time, t_{prep}.. The resulting proton transfer ratio, PTR_s is

$$PTR_s = \frac{[H]_s}{[H]_w}\eta\cdot\beta_s\cdot\lambda_s \quad [1], \qquad \text{in which} \quad \eta = \sum_{i=1}^{n} e^{\{-1+(i-1)/n\}t_{prep}/T_{1w}} \quad [2]$$

is the maximum sensitivity enhancement factor for this process. In practice, the enhancement will be less, because not all protons may exchange during t_{exch}, and label may be lost due to incomplete excitation, which is corrected for with efficiency factors for exchange transfer and labeling: $\beta_s = [1-\exp(-k_s\cdot t_{exch})]$ and λ_s, respectively. $[H]$ is the proton concentration. The factor λ_s depends on the excitation profile a pair of rectangular 90° pulses of limited bandwidth, which will be shaped as a function of $\Delta\omega_{sol}$, the frequency difference between the transmitter offset of the RF pulses and the solute protons. The profile of such an excitation can be measured and the result squared to obtain λ_s. The evolution time is varied for frequency encoding, here using a constant time (T) approach. Short 90° pulses (small hatched rectangles, 50-100μs) at an offset (o1) are used to selectively excite solute protons. The signal is acquired using a spin echo sequence with readout gradient, providing a projection of the sample in distance units for the tube we used to test the method. The magnitude of this projection is modulated as a function of evolution time due to exchange transfer, which can be reconstructed as a free induction decay of amplitude PTR_s containing signal of the exchangeable protons. A Fourier transform gives the spectrum.

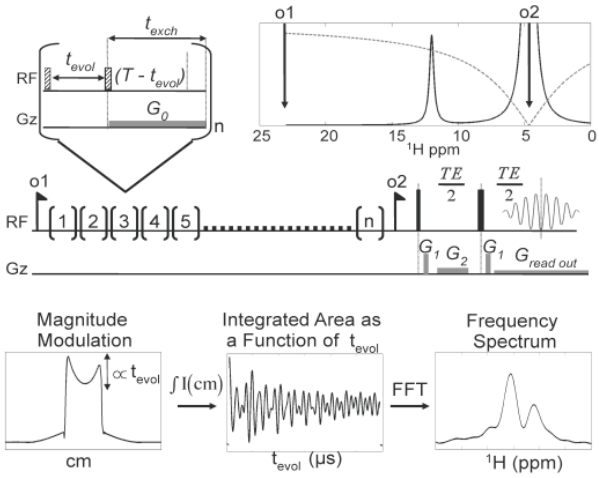

Fig. 1: Pulse sequence for frequency transfer MR

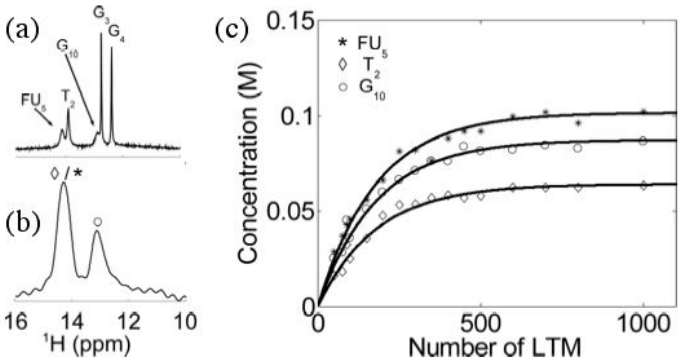

Fig. 2: Quantitative validation of frequency transfer approach for a DNA duplex: 5'-$C_1T_2G_3G_4FU_5A_6C_7C_8A_9G_{10}$-3'. (a) Jump-return spectrum with all imino protons visible. (b) frequency transfer spectra: G3 and G4 do not appear. (c) Measured concentration of solute protons as a function of number of LTMs applied; data from time domain fitting, with FU_5 and T_2 deconvolved based on rates and frequencies in the Table. Curve fits of data to eq. [1].

	Freq (ppm)	k_s(s^{-1}) [1]	β_s	λ_s [2]	$[H]_s$ (mM)
FU_5	14.38	209	0.82	0.58	1.2
T_2	14.16	81	0.49	0.56	1.3
G_{10}	13.14	304	0.92	0.45	1.2

Results & Discussion: We applied the new approach to a DNA duplex, containing several nucleotides with imino protons exchanging at different rates, including 5-Fluoro-uracil (FU), Guanine (G), and Thymine (T). We lowered the temperature to 10 °C to be able to see all protons in a conventional jump-return ($90_x/90_{-x}$ on-resonance) spectrum (Fig. 2a), allowing determination of the exchange rates from spectral linewidths ($k_s + 1/T_2^* = \pi\cdot LW$, see Table under Fig. 2). In the frequency transfer spectrum (Fig. 2b), the rapidly exchanging protons of 5-FU, T2 and G10 are visible, while slowly exchanging ones (G3, G4) are filtered out. The labeling efficiency was determined experimentally. Fig. 2c shows the results of frequency transfer spectral intensities obtained as a function of number of LTMs, and fitting according to Eqn. [1]. Using the exchange rates and efficiency parameters in Table 2, we were able to fit the solute proton concentration $[H]_s$. This was used to determine the DNA concentration of 0.6-0.65 mM, which compared well with estimated based on nucleoside analysis (0.8mM). The excellent correspondence between experimental and theoretical curve shapes provides further validation of the method.

Conclusion: We presented a novel approach to indirectly detect signals of multiple rapidly exchanging protons through the water signal using *frequency transfer* instead of *saturation transfer*. Compared to CEST MRI, FLEX MRI is expected to be less sensitive to B_0 inhomogeneity and interference by slow magnetization transfer processes due to the opportunity for time domain removal of water signals and the capability for exchange rate filtering, respectively.

References: (1) Ward, K.M.; Aletras, A.H.; Balaban, R.S. *JMR.* **2000**, *143*, 79-87. (2) Zhou, J.; van Zijl, P. C. *Progr. NMR Spectr.* **2006**, *48*, 109-136. (3) Sherry, A.D.; Woods, M. *Ann. Rev. Biomed. Engin.* **2008,** *10*, 391-411. (4) Aime, S.; Castelli D. D.; Crich S. G.; Gianolio E.; Terreno E. *Acc. Chem. Res.* **2009**, 42, 822-831.