IMAGING OF AMIDE PROTON TRANSFER (APT) AND NUCLEAR OVERHAUSER EFFECT (NOE) USING CHEMICAL EXCHANGE ROTATION TRANSFER (CERT)

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Target audience: Investigators studying tumors or other tissues using CEST methods.

Purpose: Chemical exchange saturation transfer (CEST) is an MRI technique that can indirectly detect labile protons through changes in the water signal. CEST Z-spectra show dips from exchangeable sites (-NH_2, -NH, and OH) at down field frequencies and upfield spectral features have been interpreted as Nuclear Overhauser Enhancement (NOE) (Fig. 1c). Quantitative mapping of such effects is difficult as conventional asymmetric analyses include contributions from both exchangeable sites and NOE. An alternative approach is to quantify amide and NOE effects by fitting the direct water effects[1,2] and examining deviations from the fit. However, the fitted curves are affected by several proton exchanging sites, making interpretation difficult. We recently reported a modified method, chemical exchange rotation transfer (CERT), which can quantify amide proton transfer (APT) through subtraction of CEST signals at two irradiation flip angles instead of two frequency offsets, which potentially provides a more specific amide signal[3]. Here, we demonstrate the application of CERT to *in vivo* mapping of APT and NOE in a 9L glioma in rat brain at 9.4 T.

Methods: CERT MTR_{double} is calculated by the subtraction of signals after pulse-train saturation at two net nutation angles (but constant power), while conventional CEST MTR_{asym} comes from the subtraction at two frequencies:

$$MTR_{double} = (S_-(2\pi) - S_-(\pi))/S_0\big|_{B_{avgpower}} \quad (1) \qquad MTR_{asym} = (S_+ - S_-)/S_0 \quad (2)$$

The new CEST metric MTR_{double} isolates the rotation contribution, avoids acquisitions at multiple frequencies, and avoids the confounding signals on the other side of water. Amides are a relatively slowly exchanging site, which increases the MTR_{double} sensitivity. The NOE peak also shows slow dynamic exchange effects although there is no actual chemical exchange.

A Bovine serum albumin (BSA) phantom was prepared with a concentration of 10% (w/w) and pH of 6.4. BSA and egg white phantom experiments were performed with pulsed CEST sequences at irradiation flip angles of pi and 2pi and at $B_{avg\ power}$ of 0.2, 0.4, 0.8, and 1.6 μT. Images were acquired using a 2-shot EPI sequence with matrix size 64 × 64, field of view 32 × 32 mm and number of averages = 1. Studies of 9L tumors in rat brain *in vivo* were performed with pulsed-CEST sequences at irradiation flip angles of pi and 2pi and at $B_{avg\ power}$ of 1.6 μT. Images were obtained using 4-shot EPI with matrix size 128 × 128, field of view 32 × 32 mm and number of averages = 10. Also, a T_1 map was acquired with inversion recovery spin-echo EPI with a 64 × 64 matrix. All experiments were performed on a 9.4 T Varian system.

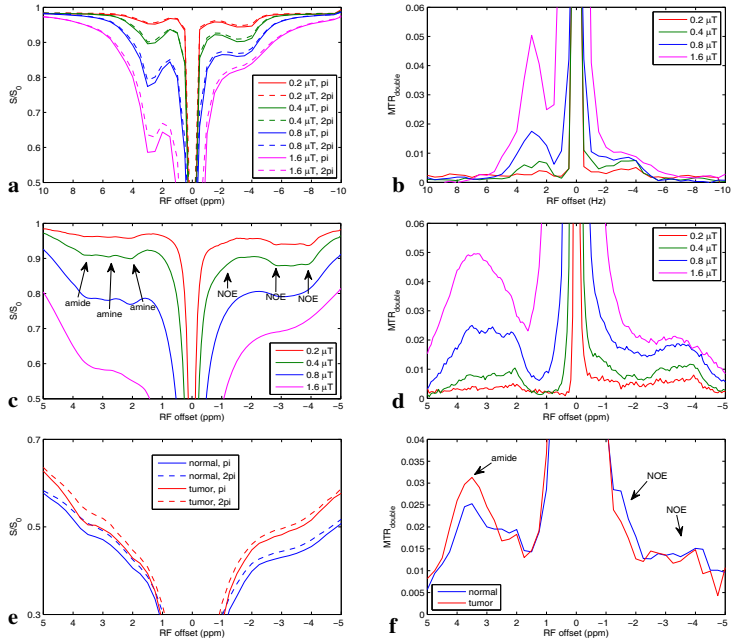

Fig. 1: Experimental Z-specta and MTR_{double} on BSA (a, b), egg white (c, d), and live rat brain with 9L tumor (e, f) at 9.4 T.

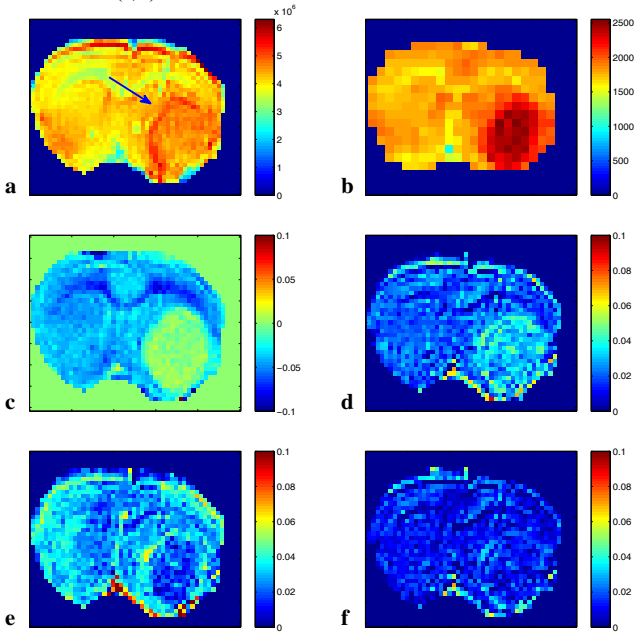

Fig. 2: (a) anatomy (T_2 weighted), (b) T_1 map, (c) MTR_{asym} (3.5 ppm), (d) MTR_{double} (3.5 ppm), (e) NOE (-1.5 ppm), and (f) NOE (-3.5 ppm) images on a rat brain with 9L tumor at 9.4 T.

Results: Fig. 1a shows z-spectra for BSA. Note the signal separation when using pi and 2pi pulses at the exchange site (2.8 ppm) and NOE (~-2-4 ppm) frequencies. This underlies the MTR_{double} results in Fig. 1b. Fig. 1c shows main dips in the egg white signal from amide at 3.5 ppm, amine at 2 and 2.75 ppm. NOEs at roughly -1, -2.75, and -4 ppm can be clearly seen at lower power. However, two NOE peaks at -2.75 and -4 ppm overlap to one peak at -3.5 ppm at higher power. The NOE at ~-1 ppm can be found by comparing the other sides of the Z-spectra and MTR_{double} at lower power. Fig. 1d plots the corresponding CERT MTR_{double} results. Fig. 1e and 1f plot the z-spectra and MTR_{double} from a rat brain bearing 9L tumor. Note that amide and the NOE at low offset (-1.5 ppm) distinguish tumor and contralateral tissues while the NOE at higher offset (-3.5 ppm) does not. Fig. 2 shows (a) anatomy imaging (T_2 weighted), showing the 9L tumor (arrow) in a rat brain, (b) T_1 map, (c) the conventional MTR_{asym} image, (d) the amide contrast (MTR_{double}) at 3.5 ppm, (e) the low offset NOE (MTR_{double}) at -1.5 ppm, showing relative lower signal in tumor core, and (f) the high offset NOE (MTR_{double}) at -3.5 ppm, showing little contrast on the ring of the tumor.

Discussion: Although good CEST contrast from tumor was found in Fig. 2c, using conventional MTR_{asym}, the main contribution is likely from the macromolecular (MT) asymmetric component, as can been seen in the WM/GM contrast. Here, we use CERT to remove this MT asymmetry. The resulting MTR_{double} shows strong amide contrast in fig. 2d. However, we do not conclude that concentration of the protein or peptides in tumor increases as this effect could be still caused by longer T_1 or a second order effect of lower MT in tumor. Spectral peaks in the NOE data are related to specific protein or metabolites and show heterogeneous contrasts in Fig. 2e and 2f.

Conclusion: We obtained *in vivo* mapping of NOE and APT through CERT. The APT effects *in vivo* in conventional CEST may be dominated by MT asymmetry rather than chemical exchange. CERT appears to produce data that is more specific to the APT. The NOE spectra show features at specific offsets that require further study for full interpretation.

References: [1] Jones CK *et al. Magn Reson Med.* 2012; 67:1579-1589 [2] Jin T *et al. Magn Reson Med*. in press [3] Zu Z *et al. Magn Reson Med*. in press.