Isolated amide proton CEST contrast at 7 T correlates with contrast–enhanced T_1–weighted images of tumor patients

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Target Audience: Physicians and physicists interested in the identification of tumor margins in MRI without contrast media and the isolation of Chemical Exchange Saturation Transfer (CEST) effects.

Purpose: Recent studies indicated that besides the nuclear Overhauser effect (NOE) of dipolar-coupled aliphatic protons upfield from water an NOE of aromatic protons resonating at the opposite side from the water peak ($\Delta\omega = 0$) is apparent in *in vivo* *Z*-spectra[1]. Hence the CEST effect of exchanging amide protons resonating at frequency offset $\Delta\omega = 3.5$ ppm includes a signal contribution from aromatic NOE. Here we propose a pure exchange-mediated contrast which is free of this contamination and thus depends exclusively on pH and the concentration of amide protons. Under the assumption that both aliphatic and aromatic NOEs *in vivo* originate from the same mobile proteins or peptides, isolation of the amide proton effect is possible by the following contrast parameter:

$$C_{EX}(\Delta\omega) = AREX(\Delta\omega) - f_{NOE} \cdot AREX(-\Delta\omega)$$

The ratio f_{NOE} between up- and downfield NOE, is constant for the same protein composition. AREX is the exchange-dependent relaxation rate representing CEST effects corrected for direct water saturation (DS), semisolid magnetization transfer (MT), and T_1 relaxation[2,3]. The isolation of the exchange-mediated amide proton effect was studied in protein model solutions and then successfully applied to *in vivo* CEST image data of patients with glioblastoma.

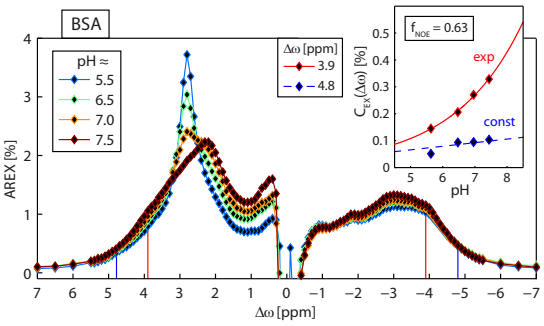

Fig. 1: AREX spectra of BSA at different pH and B_0 = 14.1 T demonstrating dependence on pH of the CEST–contrast C_{EX}.

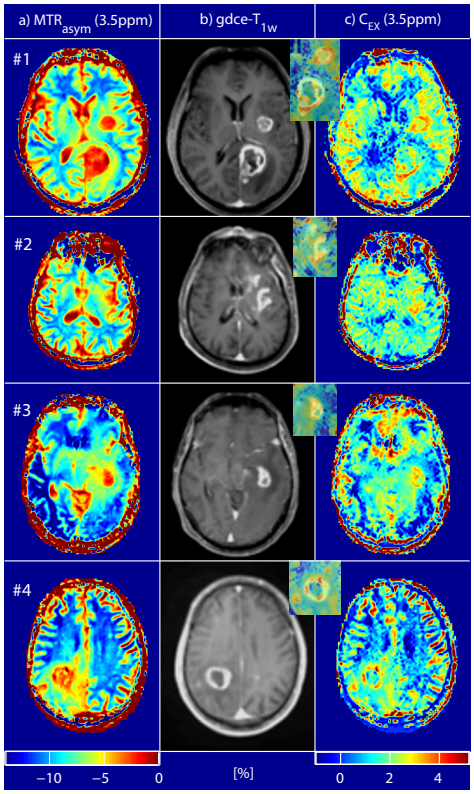

Fig. 2: Standard MTR_{asym} (a) and proposed C_{EX} (c) CEST contrast at B_0 = 7 T of amide protons resonating at $\Delta\omega = 3.5$ ppm. Fused images of the tumor region of 4 patients with glioblastoma show a distinct correlation of C_{EX} with gdce-T_{1w} (b) images.

Materials and Methods: *In vitro* CEST spectroscopy was performed with 2.5 % (w/v) aqueous bovine serum albumin (BSA) at T = 25°C and different pH on a 14.1-T NMR spectrometer (Bruker, Germany). *Z*-spectra were obtained by means of continuous wave (cw) saturation (rectangular pulse: amplitude B_1 = 0.75 µT, width t_{pulse} = 12 s). Normalized *Z*–spectra were unevenly sampled at 117 different frequency offsets. $AREX = (1/Z - 1/Z_{ref})/T_1$ was calculated using a Lorentzian fit of DS for Z_{ref}.
In vivo CEST MR imaging was performed on a 7-T whole–body MR tomograph (MAGNETOM 7 T; Siemens Healthcare, Germany) using a 2D GRE sequence. These examinations were part of an ongoing MRI study of 12 patients with newly diagnosed and histologically proven glioblastoma. CEST images were obtained after selective saturation by a train of 150 Gaussian–shaped rf pulses (t_{pulse} = 15 ms, t_{delay} = 10 ms, t_{sat} = 3.75 s, B_1 = 0.6 µT, 65 unevenly distributed offsets). AREX and MTR_{asym} were calculated by fitting the *Z*-spectra with a 5-pool Lorentzian model which yields a labeled (Z_{lab}) and a reference *Z*–spectrum (Z_{ref}) for each CEST effect. Both contrasts were corrected for B_1-inhomogeneities[4]. The 2D CEST images were co-registered with 3D T_1-weighted gadolinium contrast-enhanced images (gdce–T_{1w}) obtained at 3T.

Results: In BSA model solutions the amide proton resonance is shifted from 3.5 ppm to 3.9 ppm in the AREX spectra, presumably due to the dominant amine proton effect at 2.7 ppm. C_{EX} showed an exponential dependence on pH which corresponds to expectation for an isolated exchange–mediated CEST effect (Fig. 1, red line). In contrast, an approximately constant signal upon pH variation was observed for C_{EX} at $\Delta\omega = 4.8$ ppm where no exchange-mediated effects are apparent (Fig. 1, blue line). The value $f_{NOE} = 0.63$ was chosen such that the exponential fit as a function of pH yielded $C_{EX}(3.9\text{ ppm}) = 0$ at pH = 0. Due to the different composition of proteins *in vivo* the value of f_{NOE} was heuristically set to 0.2, resulting in a positive C_{EX} contrast across the whole image. Hyperintense regions of gdce-T_{1w} images correlate well with hyperintense regions of C_{EX} images, while MTR_{asym} shows hyperintensity over the entire tumor region (Fig. 2). Especially in patient #2 MTR_{asym} does not delineate the structure of tumor and necrosis as good as the C_{EX} contrast. Fused gdce-T_{1w} and C_{EX} images (Fig. 2b and c) illustrate the accurate overlay of the ring enhancements. Moreover, cerebrospinal fluid and brain stem, which are hyperintense in MTR_{asym} due to the absence of any CEST effect, are hypointense in C_{EX} in all examined patients. Similar results are observed in the other patients of the patient group (n = 12).

Discussion: The presented BSA data suggest an exponential dependence of C_{EX} on pH. Furthermore the AREX approach causes C_{EX} to scale linearly with the concentration of amide protons. The weak influence of pH on NOE effects (Fig. 1) allows to assume that the ratio f_{NOE} is essentially constant in different physiological environments. The remaining weak contrast between gray (GM) and white brain matter (WM) in the C_{EX} images (Fig. 2c) can presumably be attributed to different protein composition, leading to a changed f_{NOE} value, or protein concentration. Besides pH and concentration also the dependence of C_{EX} upon protein folding states should be considered[5].

Conclusion: The *in vivo* CEST effect of amide protons at the surface of mobile proteins obtained at 7 T was isolated from the underlying aromatic NOE using a simple assumption. This leads to a contrast that should be solely dependent on pH and amide proton concentration and shows very good agreement with the ring enhancement resolved in gdce-T_{1w} images from patients with glioblastoma. This is promising for a Gd-free brain cancer MRI protocol and also interesting for detection of tumor without disruption of the blood brain barrier.

References: [1] Jin, T., Kim, S.-G., Proc 21st ISMRM, p. 2528, (2013) [2] Zaiss, M. *et al*., NMR Biomed. **27**, 240-252 (2014). [3] Xu, J. *et al*., NMR Biomed. **27**, 406-416 (2014). [4] Windschuh, J. *et al*., Proc. 22nd ISMRM, p. 3303 (2014). [5] Goerke, S. *et al*., Proc. 22nd ISMRM, p. 3326 (2014).