Supporting Information

Fast Tissue Characterization with Quantitative High Resolution Magic Angle Spinning (HRMAS) CEST Z-Spectroscopy

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MATERIALS AND METHODS

Phantom

We prepared Choline (10 mM) and Creatine (10, 20, 30 40 and 50 mM) solution in copper sulfate-doped (0.65 mM) phosphate-buffered saline (PBS, Sigma Aldrich, St Louis, MO) with 1.5% agarose gel. The solution was loaded into a 4 mm Zirconia rotor with 1.0 µl of deuterium oxide (D_2O). The phantom was then solidified under room temperature before introduction into the High Resolution Magic Angle Spinning (HRMAS) probe.

Tissue Samples

All experiments were approved by the institutional animal care and use committee. Unilateral stroke was induced in four adult male Wistar rats (Charles River Laboratory, Wilmington, MA) following a standard intraluminal middle cerebral artery occlusion (MCAO) procedure under anesthesia with 2.0-2.5% isoflurane/air mixture. Briefly, a midline neck incision was made and the right common carotid artery (CCA) was carefully exposed. A 4-0 silicon-coated nylon suture (Doccol Copr., Sharon, MA) was inserted into the lumen of the right internal carotid artery, and then advancing it to block the origin of the middle cerebral artery (MCA). 24 hours after MCAO, brain tissue samples from contralateral normal area or ipsilateral ischemia lesion were harvested. Approximately 15-20 µl of each tissue sample was loaded into 4 mm Zirconia rotors with 1.0 µl of D_2O, and introduced into the HRMAS probe.

NMR

NMR measurements were carried out on a Bruker AVANCE spectrometer (Bruker BioSpin, Billerica, MA, USA), operating at 600 MHz (14.1T). For method validation, spectra from Choline/Creatine gel were acquired at 4 °C and at a HRMAS spinning rate of 3600 Hz using a spin-echo sequence without (B_1=0 μT) or with (B_1=1, 1.5, 2 and 3 μT) RF saturation. The RF saturation was achieved by applying a continuous wave (CW) irradiation on water resonance for 6s and off-resonance frequency offsets were simultaneously encoded by a gradient applied during saturation. Each spectrum has a spectral width of 9,600 Hz (16 ppm) with a total of 256 spectral points. The repetition time (TR)/echo time (TE) was 12 s/25 ms, number of average (NSA)=4, and the total scan time was 48 s. The spectra acquired without saturation provided a one-dimensional projection of the sample of interest. Spatial encoding of the off-resonance frequencies was achieved by applying a gradient along the axis of HRMAS rotation during RF saturation. Spectroscopic measurements of *ex vivo* tissue were carried out at 37 °C and at a HRMAS spinning rate of 4800 Hz. Z-spectra were acquired using a spin-echo sequence without (B_1=0 μT) or with (B_1=1, 1.5, 2μT) RF saturation. These spectra were acquired using fast spinning rates of 3600 and 4800 Hz, respectively to eliminate any contamination from spinning side-band effects.

Data Analysis

Conventional HRMAS spectroscopic data were processed using Nuts software (Acorn NMR, Livermore, CA, USA). All free induction decays were subjected to Fourier transformation, baseline correction, and phase adjustment. The resonance intensities reported here represent integrals of curve-fittings with Lorentzian-Gaussian line-shapes. A ratio between the intensities of major peak of Creatine (3.0 ppm) and Choline (3.2ppm) was derived for each sample.

Z-spectra were calculated by normalizing the spectrum acquired with saturation pulse (I) to that acquired with the saturation pulse turned off (I_0). Conventional CEST asymmetry was calculated as CESTR = (I_{ref} - I_{label})/I_0. Multi-Lorentzian fitting of the Z-spectra was used to estimate the CEST effects from different proton pools[1,2]. Briefly, the Z-spectrum was fitted as the sum of multiple Lorentzian functions with the following equation

$$1 - \frac{I}{I_0} = \sum_{i=1}^{N} \frac{A_i}{1+4\left(\frac{\omega-\omega_i}{\sigma_i}\right)^2} \quad [1]$$

where ω is the frequency offset from the water resonance, A_i, ω_i and σ_i are the amplitude, frequency offset and linewidth of the CEST peak for the i^{th} proton pool, respectively. In the CEST phantom, we employed a simple two-pool Lorentzian model, including water (0 ppm) and creatine (1.9 ppm). For the brain tissue samples, a multi-pool Lorentzian model of water (0 ppm), MT (-2 ppm), amide (3.5 ppm), amine (2 ppm) and Nuclear Overhauser enhancement (NOE) effects (-3.5 ppm) as well as 4.7, 2.8, -1.25 and -2.5 ppm, which showed distinguishable CEST effects was used.

Table S1. Longitudinal relaxation time T_1 and transverse relaxation time T_2 of tissue samples from contralateral normal area or ipsilateral ischemic lesion at 600MHz and 37 ºC. Mean ± standard deviation are presented.

	T_1/s	T_2/ms
Normal	2.68±0.35	37.9±4.2
Ischemic	3.37±0.53	52.3±6.8
p-value	0.018	0.049

Figure S1 a) Lorentzian decomposition of a representative HRMAS Z-spectrum (B_1= 1 μT) from a gel phantom containing 10 mM Choline (Cho) and 30 mM Creatine (Cr). The residuals computed between the sum of the fitted peaks and the original data was shown in the bottom. b) The amplitude of the fitted Cr peak also showed a strong linear correlation with Cr concentration (R^2=0.985, P=0.0008).

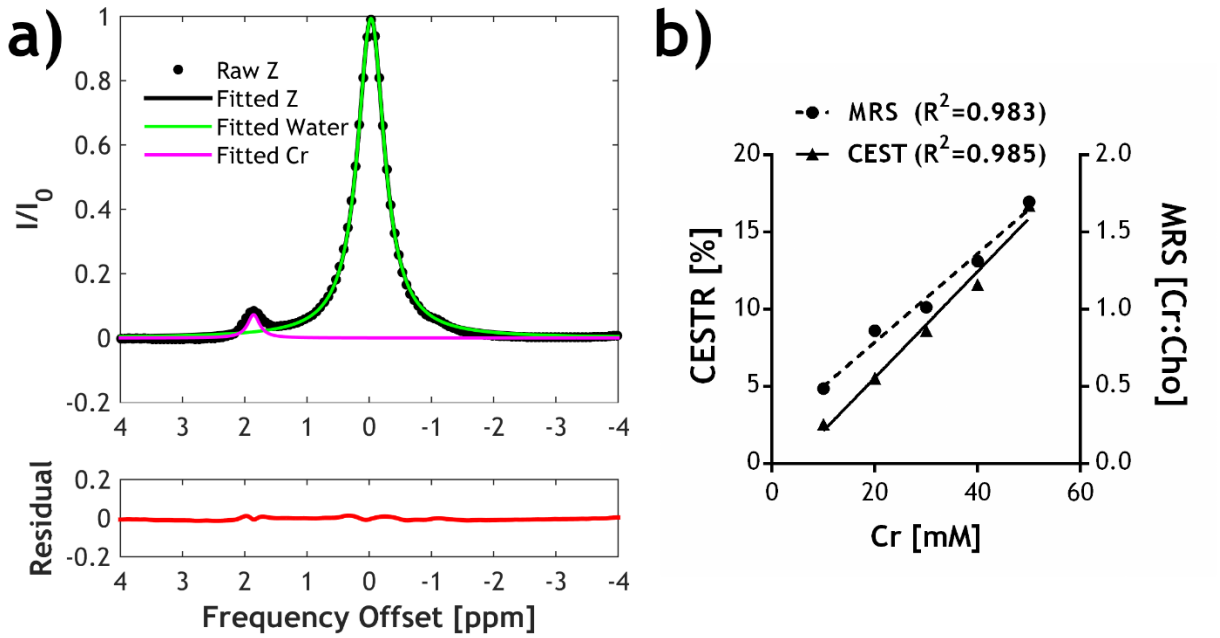


Figure S2 Multiple CEST peaks can be clearly observed after subtracting the fitted water and MT effects from the Z-spectrum (B_1= 1 μT) of normal brain sample.

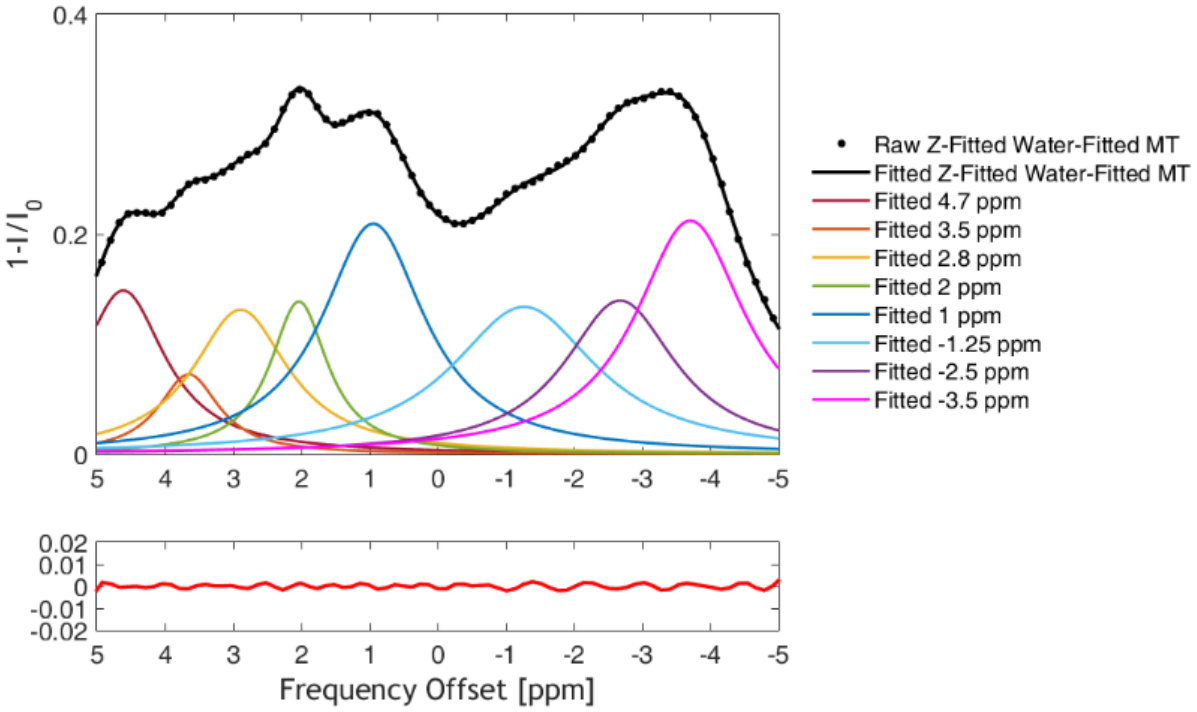


Figure S3 Multi-Lorentzian fitting of HRMAS Z-spectrum (B_1= 1, 1.5 and 2 μT) from **a**) normal brain tissue or **b**) ischemic tissue. The residuals computed between the sum of the fitted peaks and the original data were less than 1%, indicating a good fitting quality.

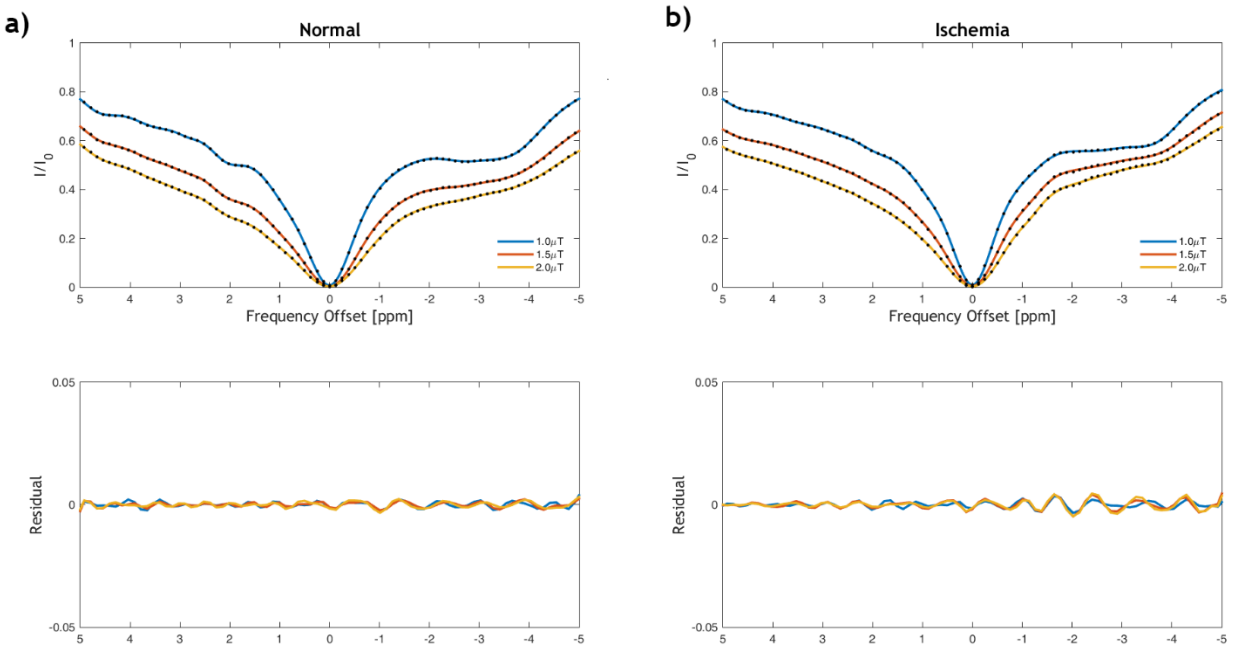
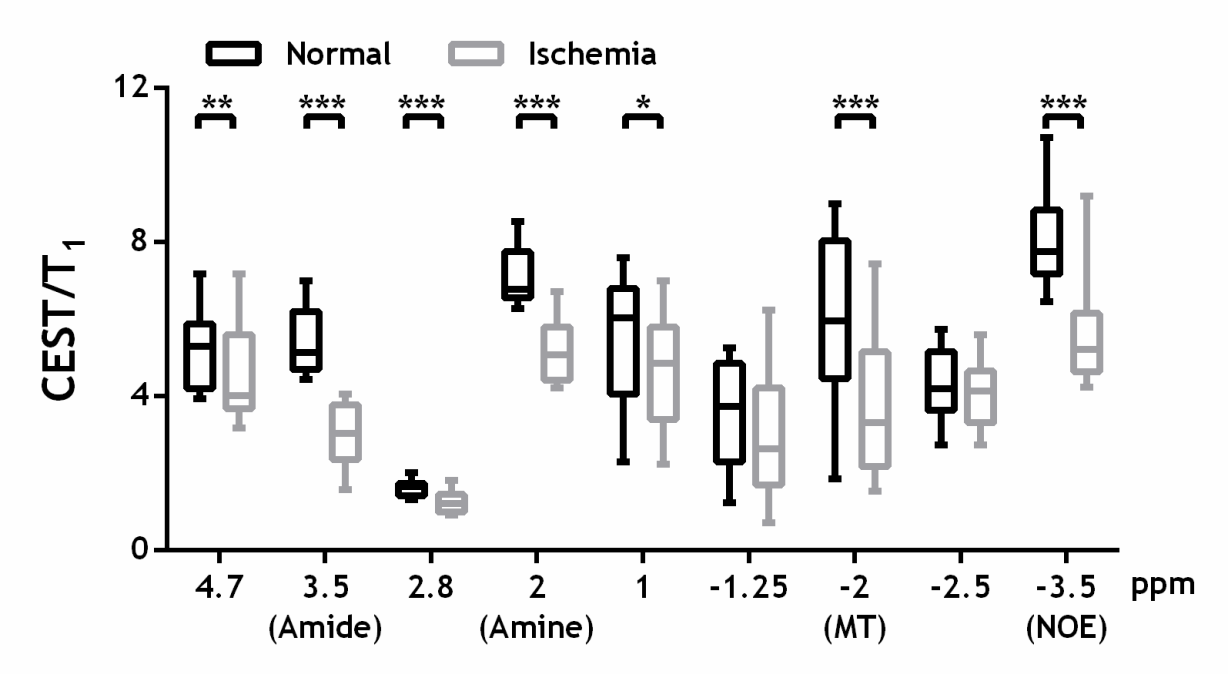


Figure S4 Boxplot of T_1-corrected amplitudes of fitted CEST peaks in the HRMAS Z-spectra from normal and ischemic tissue samples (N = 4 animals × 3 powers). Paired Student's t-test was performed with *P<0.05, **P<0.005 and ***P<0.001.



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