

High throughput MRI method for *in vitro* CEST agent screening

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INTRODUCTION Chemical Exchange Saturation Transfer (CEST) has recently emerged as a novel MRI contrast mechanism¹ with great promises. However, the conventional CEST NMR method is too slow for high throughput screening and characterizing libraries for new CEST contrast agents, due to extremely long acquisition times required to collect multiple spectra for a z-spectrum analysis². The sample throughput is also limited because high resolution NMR spectrometers are intrinsically designed to handle a single uniform sample. Although multiplex NMR has been demonstrated, special hardware and sample preparation are typically required to maintain sensitivity and, especially, B₀ field homogeneity.^{3,4} The goal of this study is to show that high throughput MRI for screening CEST agents is possible even when using multiple samples in a magnetically inhomogeneous environment. The reason is that the width of the saturation spectrum is not affected by T2* but by T2, while the susceptibility shifts can be corrected for on a voxel-by-voxel basis.

METHOD AND MATERIALS Protamine sulfate (Sigma-Aldrich), myo-inositol (Sigma-Aldrich) and 16 peptides (12-residue long each, Bio S&T Inc) were dissolved in 10 mM PBS to desired concentrations (protamine sulfate: 0.5 mg/ml to 5 mg/ml, myo-inositol: 31.25mM, non-arginine-containing peptides: 2.5 mg/mL, and arginine-containing peptides: 1.25 mg/ml). The pH of each sample was 7.3, unless otherwise noted. All samples were placed in 1 mm capillary tubes with a volume of approximately 50 μ l. The capillaries then were arranged on a 18-well MR compatible homemade tube holder (Fig 1A). All MRI images were acquired at 310K using an 11.7T Bruker Avance system equipped with a 15 mm birdcage RF coil. The main field (B₀) was manually shimmed to second order, or purposely deshimmied by adjusting the X, Y and Z' shim gradients. A modified RARE (TR=6.0 sec, effective TE= 4.3 ms, RARE factor =16, slice thickness=0.5 mm, and NA=2) including a magnetization transfer (MT) module (one CW pulse, (ω_1) = 4.7 μ T (200 Hz), 4sec) was used to acquire CEST weighted images. The B₀ inhomogeneity was assessed using the WAtER Saturation Shift Reference (WASSR) method,⁵ in which the absolute water frequency in each voxel is measured using direct water saturation. The WASSR pre-scan was conducted prior to CEST imaging with identical image readout (except TR=2 sec) but a short, weak saturation pulse (t_{sat} = 200 m μ s, ω_1 =0.5 μ T (21.3 Hz)) sweeping from -2ppm to 2 ppm (0.1ppm step). A point-resolved spectroscopy (PRESS) sequence was used to acquire MR spectra under different shimming conditions (13x13x9.5 mm³ or 0.5x0.5x0.5 mm³, TR/TE = 1000/16.7 ms, NA=512, spectrum acquisition size =8192, spectral sweep width =10,080 Hz). All data processing was performed using custom-written scripts in Matlab. Frequency of all voxels in each CEST-weighted image was corrected using the WASSR determined water frequencies, after which the asymmetry in the magnetization transfer ratio (MTR_{asym}) was determined to measure the CEST effect⁵

RESULTS AND DISCUSSION Figs. 1A and 1B show the arrangement of small capillaries of 20 μ l to 50 μ l CEST agent solutions put together in an anisotropic arrangement in a sample holder. There was no liquid in between the tubes, making it almost impossible to achieve acceptable magnetic field homogeneity. The acquisition time per sample was 2.6 minutes with saturation frequencies ranging from -7 ppm to +7 ppm (step=0.2 ppm). Much time is saved by measuring 18 samples instead of one. Due to the high sensitivity of CEST, the significant reduction in sample volume does not compromise the SNR of CEST study.

The presence of many high-density glass-air interfaces leads to a large variance in B₀ across the sample, as is clear from the field frequency map in Fig. 1C, resulting in mis-registration of the frequency for each pixel which can either reduce real contrast or generate false contrast.⁶ Without spatial encoding, the waterline would be broad and asymmetric and even with spatial encoding shimming cannot be optimized for all tubes. However, the width of saturation spectra depends only on T2, not T2* allowing high quality CEST spectra to be obtained in each voxel. These Spectra are shifted between voxels, but we employed the WASSR procedure (explained above) to determine the absolute water resonance frequency on a voxel-by-voxel basis, and used this to correct the center frequency of each CEST spectrum. The WASSR procedure was able to provide satisfactory B₀ correction for both well-shimmed samples and severely deshimmied samples, and produced identical MTR_{asym} maps for all samples

Fig. 2 shows application of the high-throughput approach to a library of 16 short peptides with different amino acid sequences. Pixel by pixel analysis of the CEST contrast (MTR_{asym}) reveals that each peptide has a specific CEST profile or CEST 'finger print' that can be used identify and distinguish it from other polypeptides.

CONCLUSION This study demonstrates a new high throughput MRI method for simultaneously screening multiple CEST contrast agents *in vitro*. The proposed method is fast and able to provide high-quality CEST spectra even in presence of severe B₀ inhomogeneity induced by bulk magnetic susceptibility effects. This method has the potential to greatly facilitate the development of CEST contrast agents as well as screening libraries of new drugs and enzymatic reactions.⁷

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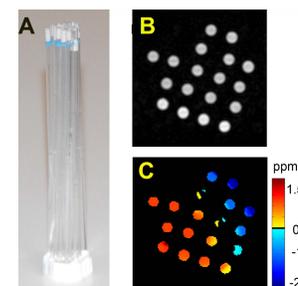


Figure 1. A) High throughput sample holder for a vertical MRI scanner, B) the axial T₂weighted MRI image and C) water frequency map reflecting the B₀ inhomogeneity

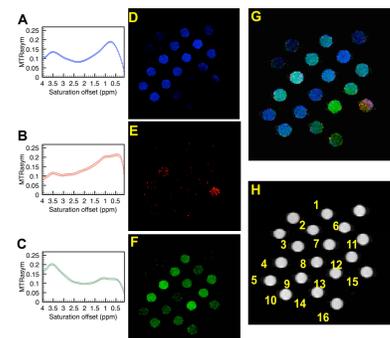


Figure 2. Screening CEST properties (A-C) of 16 short peptides allows constructing a library with CEST 'finger print' assigned to each individual peptide (D-H). A-C: the CEST features of (DSSS)3, (DTTTT)2, and (RTTT)3. D-F: CEST images at 0.8, 1.8 and 3.6 ppm. The polypeptides were labeled as in T2weighted image (H) 1:(KS)6, 2: (KSSS)3, 3: (DSSS)3, 4: (DSSSS)2, 5: (DTT)4, 6: (DTTT)3, 7: (DTTTT)2, 8: (ETT)4, 9: (ETTT)3, 10: (ETTTT)2, 11: (TK)6, 12: (TTK)4, 13: (TTTK)3, 14: (TTTTTK)2, 15: (RT)6, 16: (RTTT)3. K = lysine, S = Serine, R = Arginine, T = Threonine, E = Glutamate, D = aspartate