Amine/Amide Concentration Independent Detection (AACID) of Intracellular pH by CEST MRI at 9.4T

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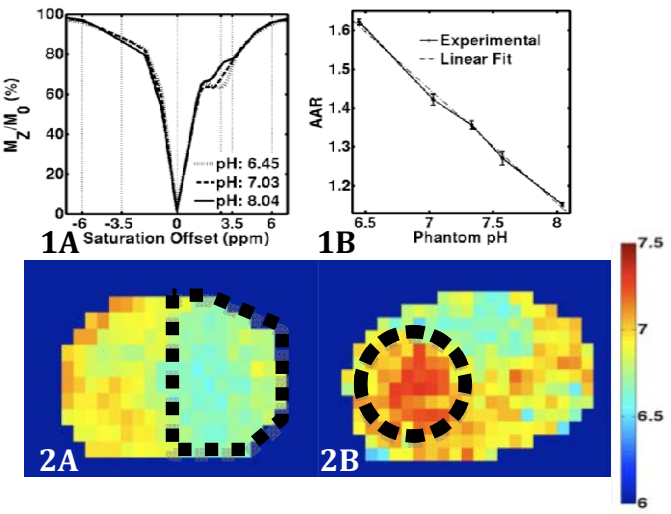
Target audience: Physicists and chemists developing novel magnetic resonance imaging (MRI) contrasts. Scientists using chemical exchange saturation transfer (CEST) or amide proton transfer (APT) MRI contrasts to measure intracellular pH (pH_i) in vivo.

Purpose: Zhou et. al first used exchanging amide protons (resonating at ~3.5ppm) associated with endogenous intracellular proteins to measure pH_i *in vivo*[1]. Recently, Jin et. al. reported significant pH_i-dependent amine proton exchange (APEX) contrast at ~2.75ppm[2]. Desmond et al. recently proposed a ratiometric approach to measure pH using amide (3.5ppm) and amine (2.0pmm) CEST effects[3]. In the current study, a novel ratiometric technique is presented to directly measure absolute pH_i using the ratio of CEST contrast from amine (~2.75 ppm) and amide (~3.5 ppm) protons: amine/amide concentration independent detection (AACID)

Methods: **Amine/Amide Ratio (AAR)**: A novel expression (Eq. 1) was used to measure the ratio of amine and amide CEST effects, where $M_Z(\omega)$ is the MRI signal intensity measured following a saturation pulse of frequency ω. $AAR = [M_Z(2.75\text{ppm}) - M_Z(6.00\text{ppm})] / [M_Z(3.50\text{ppm}) - M_Z(6.00\text{ppm})]$ (Eq. 1). **MRI Experiments:** All MRI experiments were performed using a 9.4 T horizontal bore Agilent MRI scanner (Agilent, Palo Alto, CA). All AARs were measured applying Equation 1 to full CEST spectra (3 averages) following B_0-correction. CEST spectra were acquired using a 4 sec, 1.5 μT continuous wave radiofrequency (RF) saturation pulse and a standard fast spin echo (FSE) pulse sequence (TR/TE = 7000/7 ms, ETL=32, ETE=10ms, matrix= 64x64, FOV=25.6x25.6mm^2, 2 prescans, slice thickness=2mm). ***In vitro:*** Phantoms containing 10% (by weight) bovine serum albumin (BSA) dissolved in phosphate buffered saline (PBS) were produced with varying pH (6.47, 7.05, 7.32, 7.57, 8.05). AARs were calculated for each pH phantom based on acquired CEST spectra. ***In vivo* AAR-pH_i Calibration:** *In vivo* and post-mortem AARs were measured in normal healthy mice (n=3) followed by pH_i measurement using ^{31}P-MRS using previously described methods[4]. ***Stroke & Tumor pH_i Mapping***: Focal ischemia was induced by permanent intraluminal filament occlusion of the left middle cerebral artery (MCA) in mice (n=3) as described previously[5]. pH_i-maps were acquired within 2-5 hours post-surgery for the stroke mice. Glioblastoma multiforme brain tumors were induced in NU/NU mice (n=3) as described previously[6]. pH_i maps were acquired approximately two weeks following tumor cell injection for cancer mice. Standard T_1, T_2 and diffusion-weighted images were acquired to identify the anatomical changes in all stroke and tumor mice.



Results: Figure 1A shows CEST spectra from 10% BSA phantoms with different pH. The amine/amide ratio measured using CEST spectra from 5 different pH phantoms is shown in figure 1B. Eq. 2 defines the AAR-pH_i relation incorporating the *in vivo*/post-mortem ^{31}P-MRS calibration (data not shown). $pH_i = -4.50 * AAR + 12.50$ (Eq. 2) pH_i maps were calculated in stroke and cancer mice using Eq. 2. Figures 2A and 2B show pH maps from a stroke mouse and cancer mouse respectively. The dashed line represents the location of the ischemic/tumor regions based on the T_1, T_2, and diffusion weighted images (images not shown).

Discussion: *In vitro* results show that AAR decreases linearly with increasing pH_i within a physiological range (pH=6.0-8.0) independent of concentration. A linear AAR pH-dependence allowed a practical *in vivo*/post-mortem pH-calibration using ^{31}P-MRS. High-resolution pH_i maps were acquired in stroke and tumor mouse models. Measured pH_i values in normal (~6.9-7.1), stroke (~6.5-6.8) and tumor (~7.3-7.5) regions all agree with previously reported values [1,7]. A 4 sec, 1.5μT saturation pulse allowed selective saturation of the relatively slow exchanging amine protons associated with proteins and ensured that the contribution of intermediate exchanging amine protons associated with amino acids (ie. glutamate) was negligible as previously discussed[2]. The novel AAR also avoided measuring NOE effects around ~-3.50ppm which have also recently been shown to be pH-sensitive[8].

Conclusion: A novel ratiometric approach was developed to measure pH_i based on the CEST effects from amine and amide protons associated with endogenous proteins. This novel study represents a significant improvement over current methods used to quantify intracellular pH.

References: 1. Zhou et al. *Nature*. 9(2003): 1085-1090 2. Jin et al. *NeuroImage*. 2(2012): 1218-1227. 3. Desmond et al. *OctoberCEST Workshop*. MA, USA (2012). 4. Moon et al. *The Journal of Biological Chemistry*. 248(1973): 7276-7278. 5. Belayov et al. *Brain Research*. 833(1999): 181-190. 6. Li et al. *Magnetic Resonance in Medicine*. 66(2011): 67-72. 7. Webb et al. *Nature*. 11(2011): 671-677. 8. Jones et al. *OctoberCEST Workshop*. MA, USA (2012)