# A NOVEL CEST-MRI RATIOMETRIC APPROACH FOR IN VIVO PH IMAGING

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**Target audience:**
Those interested to exploit in vivo MRI pH measurements

**Purpose:**
MRI-CEST responsive contrast agents are probes able to report on physico-chemical parameters of diagnostic interest; however, their responsiveness should be made concentration independent for in vivo measurements. This task is currently achieved by using CEST agents containing multiple sets of magnetically non-equivalent protons [1]. Unfortunately, only a limited number of CEST systems fulfill this condition [2,3]. Moreover, the difference in the chemical shift between the resonances has to be sufficiently large for their selective labeling [4]. In the present study we propose an improvement of the standard ratiometric approach to molecules possessing even a single set of mobile protons by exploiting the dependence of the parameter of interest from the power of the applied RF irradiation fields (B1). Thus, instead of calculating the ratio of the ST effects from two resonances in the same molecule, at the same RF irradiation power, the effect on the observed ST upon changing the RF power is exploited. We tested this novel ratiometric approach on a FDA-approved iodinated contrast medium, iobitridol (Fig. 1a), possessing only a single amide proton pool, showing its accuracy on reporting in vitro pH as well as its ability to measure pH inside kidneys and tumor tissues.

**Methods:**
ST efficiency was measured in phantoms of a 30 mM iobitridol phosphate buffer solution with pH range 5.5 to 7.9. CEST experiments were carried out on a 7T Bruker MRI scanner at 37°C using a fast spin-echo sequence preceded by CW RF irradiation saturation pulses at different intensity (1.5-3-6 μT) for 5s. The ratio of the ST effects upon irradiating the amide absorption (5.6 ppm) at two different saturation powers (1.5/3 μT and 1.5/6 μT) was used for the calculation of the pH calibration curve.
In vivo pH mapping has been performed upon iv injection of a Iobitridol solution at a dose of 1.5 g I/kg in healthy balb/c mice and of 2 g I/kg bw in a murine model of breast adenocarcinoma (TSA tumoral cell lines). The Z-spectra were acquired, before and immediately after the i.v.injection of the agent, at two different B1 powers of 1.5 and 6 μT x5s. The post-injection ST% curves were subtracted from the pre-injection ST% curves and the obtained ratiometric values were exploited to calculate pH values from the in vitro pH calibration curve.

**Results/Discussion:**
In vitro results show that the level of the STs depends on bothpH and B1 of the irradiation RF pulses (Fig. 1b). Upon irradiating the amide proton resonance at different B1 values, it is possible to set-up a new ratiometric method (Fig 1c) for a concentration independent pH assessment (Fig 1d). This novel ratiometric approach showed a high sensitivity and accuracy in pHdetermination (Fig. 1e). In addition this new approach is concentration independent (Fig, 1f). Mean renal pH values between 6.6 and 6.7 were obtained, with reasonable differentiation of the calyx-inner medulla and outer medulla-cortical regions (Figs. 1g, h) and allowed the assessment of the extracellular pH that resulted to be 6.4 ±0.4 in the murine xenografted tumor investigated in this work (Figs. 1i, l).

**Conclusions:**
The former prerequisite of the standard ratiometric approach, i.e. the presence of multiplenon-magnetically equivalent resonances on the same molecules is overcome by this new proposed ratiometric approach, which yields accurate pH determination upon irradiating only a single set of exchanging protons. This new approach widens markedly the type of molecules that can be considered for the set-up of ratiometric CEST methods.

**Acknowledgement / References:**
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