

# High Spatio-Temporal Resolution pH<sub>e</sub> Mapping of a Rat Glioma Derived From pH-Dependent Spin-Lattice Relaxivity

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## Introduction

The extracellular pH (pH<sub>e</sub>) of cancers is acidic and inhibiting this acidity will inhibit metastases. Methods to image pH<sub>e</sub> in tumors based on relaxivity of a pH-dependent contrast reagent (CR), Gd-DOTA-4AmP were developed. We investigated the single injection of a mixture of DyDOTP with GdDOTA-4AmP. The ΔT<sub>1</sub>, and ΔT<sub>2</sub><sup>\*</sup> induced by the Gd-CR exhibited similar pH-dependence, while the pH-independent Dy-CR reduced T<sub>2</sub><sup>\*</sup> with negligible effects on the T<sub>1</sub>. Thus, with calibration, co-injection of this cocktail can enable dynamic calculation of spatially localized unique pH values. One concern with this approach was the low dynamic range afforded by gradient echo T<sub>2</sub><sup>\*</sup> imaging in vivo, because of high endogenous relaxivity. We have overcome this problem using Echo Planar Spectroscopic Imaging (EPSI); a technique that generates full 1H spectra of the water resonance for each pixel at high resolution.

## Methods

The Spin-Lattice relaxivity of the CR Gd-DOTA-4AmP is sensitive to pH. CR concentration and total relaxation rate were determined simultaneously by two independent experimental observables: Spin-Spin relaxation and Spin-Lattice relaxation as shown in the protocol schematic, Figure 1. In a continuous infusion experiment, measurements were performed during co-injection of a CR cocktail composed of Gd-based CR and Dy-based CR at the molar ratio 1:2. A single radio-frequency pulse EPSI sequence is sensitive to CR-induced alterations in the in vivo magnetic microenvironment, where the change in linewidth (ΔR<sub>2</sub><sup>\*</sup>) of each spectrum is proportional to CR concentration. An in vivo calibration of ΔT<sub>2</sub><sup>\*</sup> versus Gd concentration was done in a C6 rat glioma model. The calibration part of the method (which only needs to be done once), was performed by gradually infusing pH insensitive Gd-DTPA with Dy-DOTP, and interleaving EPSI with a T<sub>1</sub>-weighted spin echo. The T<sub>1</sub>-weighted images are sensitive to Gd-DTPA, since the Gd affects the Spin-Lattice relaxation to first order, while the Dy-DOTP does not significantly affect T<sub>1</sub>. This is evident in the series of images that show enhancement of the tumor. Similarly, the Dy-DOTP has the dominant contribution to ΔT<sub>2</sub><sup>\*</sup>. The result is a correlation between ΔT<sub>2</sub><sup>\*</sup> and T<sub>1</sub>-weighted intensity, which relates back to Gd concentration. In the pH mapping part of method, pH sensitive Gd-DOTA-4AmP is co-infused with Dy-DOTP, and again monitored with interleaved EPSI and T<sub>1</sub>-weighted images. These relaxivities yield a relatively high spatio-temporal resolution pH map of the glioma. All experiments were carried out on a Bruker Biospec 4.7 T scanner, with gradients capable of 200 mT m<sup>-1</sup> strength and a maximum slew rate of 880 mT m<sup>-1</sup> s<sup>-1</sup>. EPSI was performed with two spatial dimensions without the use of water-nor outer volume suppression with trapezoidal gradients and an RF excitation was a single 90° pulse. For EPSI data of 128<sup>3</sup> points, the time resolution of a single experiment was determined by the time necessary to complete all phase encoding scans, which was 2 minutes. The resulting in plane spatial resolution was 250 μm/pt on each side with 1 mm slice thickness. The spectroscopic resolution was 3.7 Hz/pt.

## Conclusions

The EPSI derived concentrations are in a reasonable range for this contrast agent which is validated by the range of molar relaxivities within the ROI, that is located within the glioma where CA extravasates. These relaxivities yield a pH map based on a pH titration of GdDOTA-4AmP in Fetal Bovine Serum phantoms as in previous work (4, 5). The pH values within the glioma are acidic as is demonstrated in the histogram of Figure 2. A considerable methodological advance in the determination of tumor pH has been demonstrated in a rat glioma. A single injection protocol consisting of slow infusion of a CA cocktail has yielded a high resolution pH map. The primary advantage of this protocol over previous studies (1-5) is the rapidity of the pH measurement, given that the calibration curve has been obtained. This high resolution pH<sub>e</sub> map was achieved with a relatively modest concentration of GdDOTA-4AmP, which is ca. 0.20 mM (16 minutes after injection). In principle, this method is capable of yielding pH<sub>e</sub> maps within practical times in a clinical setting.

## References

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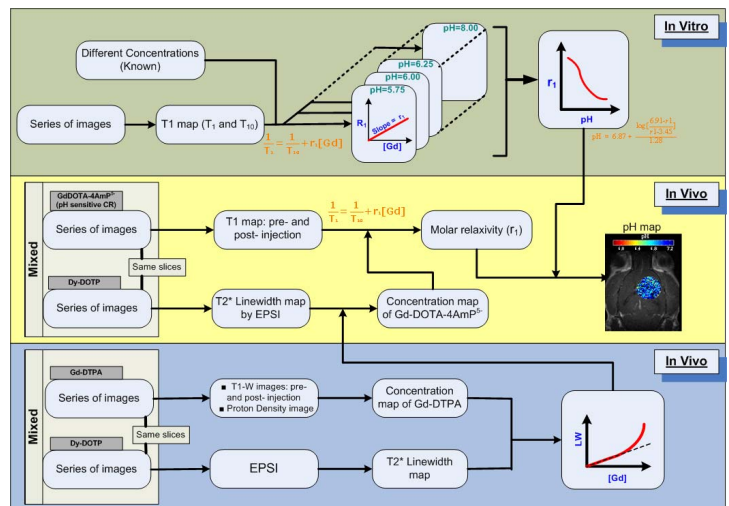


Figure 1: Schematic overview of the single injection experimental

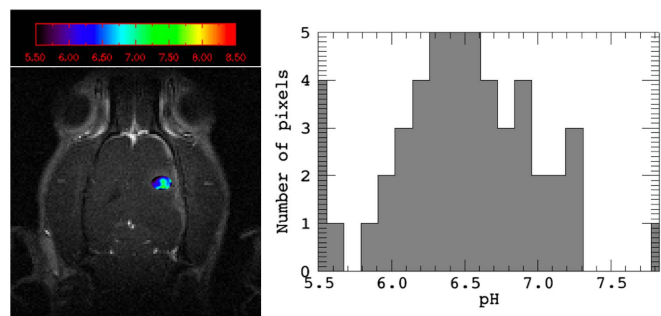


Figure 2: pH<sub>e</sub> map of tumor and responding Gd-DTPA histogram