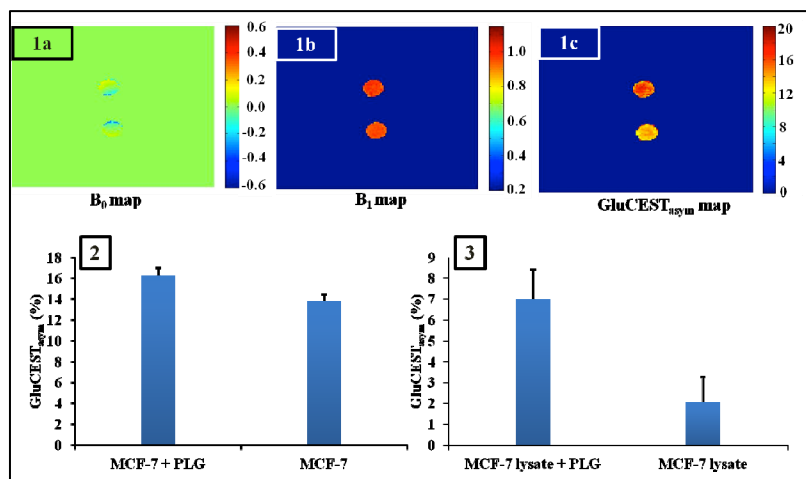# CEST imaging of human breast cancer cells

Catherine DeBrosse[1], Mohammad Haris[1], Ravi Prakash Reddy Nanga[1], Hari Hariharan[1], Damodar Reddy[1], Imran Mohammad[2], Kejia Cai[1], Anup Singh[1], and Ravinder Reddy[1]

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Introduction: Tumor progression has been linked to the high levels of proteases found within many tumor cells. Proteases such as cathepsins are responsible for protein degradation as tumors invade healthy tissue[1,2]. Novel cancer treatments utilize protease over-expression by conjugating a cancer therapy drug to polymers, which will be degraded by the tumor proteases[3]. One polymer that has been used for this conjugated drug delivery is poly-L-glutamate (PLG)[4], which, when degraded by tumor cathepsins, breaks down into glutamate oligomers. These smaller fragments contain labile amine protons (solute pool) that exchange with the water protons (solvent pool); this exchange phenomenon is exploited by glutamate chemical exchange saturation transfer (GluCEST) imaging. After degradation of PLG by cathepsin enzymes, a GluCEST signal is observed from the amine protons of individual glutamate moieties due to degradation of PLG by cathepsin enzymes, that is not present in phantoms containing PLG in absence of enzymes (unpublished results). To demonstrate feasibility of GluCEST imaging of polymer-conjugated drugs in tumors, we have examined the degradation of PLG in MCF-7 human breast cancer cell lines. We have investigated the increased ability of PLG to enter tumor cells by performing GluCEST imaging on intact cells as well as the cell lysates of MCF-7 cells. We hypothesize that PLG entry into cells and subsequent exposure to cathepsin may be the rate-limiting step.

Methods: Imaging experiments were performed using a custom small solenoidal coil on a 7T whole body scanner (Siemens Medical Systems, Erlangen, Germany), and using a small-animal coil on a 9.4T spectrometer (Varian Inc., Palo Alto, CA). *Intact cells*: MCF-7 human breast cancer cells were trypsinized and washed with sterile PBS and transferred into 5mm NMR tubes. Each sample contained ~50 million MCF-7 cells. PLG in 0.2 mL of saline solution (20 mg/mL) was added to 0.4 mL of MCF-7 cells. Cells were incubated for approximately 30 minutes. As a control, 0.2 mL of saline solution without PLG was added to a separate tube of 0.4 mL MCF-7 cells. CEST imaging was performed on the 7T system at room temperature using a saturation pulse of 1s and a $B1_{rms}$ of 2.9µT. CEST images were acquired every 9 minutes in both the control (MCF-7 cells + PBS) and the treated (MCF7 cells +PBS+PLG) samples over the course of 2 hours in order to determine reproducibility. Imaging parameters: Slice thickness = 6 mm, field of view = 40 x 40 mm^2, matrix size = 128 x 128. *Cell lysate:* MCF-7 cells were washed with sterile PBS and lysed in RIPA buffer. PLG in 0.1mL of saline solution (20mg/mL) was added to 0.3 mL of MCF-7 cells and allowed to incubate for 30 minutes before imaging at 9.4T. CEST images were acquired for a control sample containing 0.3 mL MCF-7 cells + 0.1 mL of saline solution immediately following the PLG treated sample. Imaging parameters: Slice thickness = 3 mm, field of view = 30 x 30 mm^2, matrix size = 128 x 128.

Data analysis: Image processing was performed using in-house MATLAB scripts. B_0 and B_1 maps were used to generate corrected CEST images as described previously[5]. GluCEST contrast was computed by subtracting the normalized magnetization signal at the glutamate proton frequency ($\Delta\omega$ = +3 ppm), from the magnetization at the corresponding reference frequency symmetrically at the opposite side of the water resonance ($-\Delta\omega$), according to the following equation.

$$CEST_{asym}(\Delta\omega) = \frac{M_{sat}(-\Delta\omega) - M_{sat}(+\Delta\omega)}{M_{sat}(-\Delta\omega)}$$

Results and Discussion: When added to MCF-7 cells, PLG degrades into glutamate fragments, exposing labile amine protons that give a GluCEST signal. GluCEST maps from two 5mm NMR tubes containing MCF-7 cells + PLG (**top**) and MCF-7 cells without PLG (**bottom**) are shown in **Figure 1.** The B_0 map (**Figure 1a.**) and B_1 map (**Figure 1b.**) are used to generate the corrected CEST map (**Figure 1c.**). **Figure 2** shows average values of MCF-7 cells both with and without PLG. There is a ~10% increase in the cells treated with PLG, indicating that the polymer is degraded by cathepsins in the tumor. In the cell lysate, a very low baseline GluCEST (~2%) is observed in the control sample, indicating that there is very little free glutamate present. When PLG is added to the cell lysate, a large increase (~3 fold) in GluCEST signal is observed (**Figure 3**). This could indicate that cell lysis exposes a large amount of the tumor cathepsins. This facilitates PLG degradation and results in the very high increase in GluCEST signal. Future work will include *in vivo* animal studies to validate the use of GluCEST for imaging of tumors, with the potential to observe targeted drug delivery through use of PLG conjugates.

References: [1] Jedeszko, C. et al. *Biol. Chem.* 385(2004): 1017–27 [2] Rochefort, H. *Acta. Oncol.* 31(1992): 125-30 [3] Melancon, M.P., et al., *Pharm. Res.* 24(2007): 1217–24 [4] Li, C., et al. *Adv. Drug. Deliv. Rev.* 54(2002): 695-713 [5] Cai, K., et al., *Nat. Med.* 18(2012): 302-6