AN ENZYME-RESPONSIVE PARACEST MRI CONTRAST AGENT THAT "TURNS ON" AFTER CATALYSIS

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INTRODUCTION: Transglutaminase (TGase) is an important biomarker of tumor vascular normalization that can cross-link extracellular matrix proteins by coupling lysine and glutamine side chains [1]. A T1 MRI contrast agent has been used to detect TGase by monitoring the accumulation of the agent in tumor tissue, although this approach lacks specificity for enzyme activity [2]. For comparison, CEST MRI contrast agents can detect enzyme activities with good specificity by measuring a change in CEST caused by cleavage of a specific bond of the agent by an enzyme [3-8]. We have designed a paramagnetic CEST (PARACEST) agent, consisting of Tm(III) chelated with 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid-10-cadaverine (Tm-DO3A-cadaverine), to detect the activity of TGase. Unlike most other enzyme-responsive CEST agents that show a disappearance of CEST after enzyme *cleavage* of a covalent bond, Tm-DO3A-cadaverine is designed to show the appearance of CEST after the *creation* of a covalent bond by TGase.

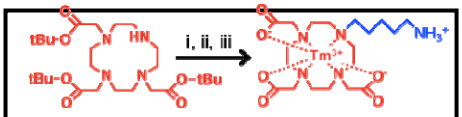

Figure 1. Synthesis of Tm-DO3A-cadaverine. i. tButyl-DO3A [6], bromopentylamine, K_2CO_3, 24 h; ii. 70% TFA, 2 h iii. $TmCl_3$, 70°C, 48 h

METHODS: **Synthesis:** DO3A was alkylated with bromopentylamine, and the protecting groups were removed (Fig. 1). Chelation was performed with 2.6 equivalents of Tm(III), and excess Tm(III) was removed by forming a precipitate after raising the pH to ~9.0. **Enzyme Reaction:** 0.25 units of recombinant microbial TGase (Zedira GmbH) was incubated with 30 mM of Tm-DO3A-cadaverine, 80 mM of the peptide Cbz-Gln-Gly (ZQG), and 40 mM of glutathione in tris-HCl buffer at pH 7.4 and 37°C for 18 hours. Reverse-phase HPLC with a 30-minute solvent gradient of 0-30% acetonitrile in water was performed before and after the reaction. CEST spectra were also acquired before and after the reaction. **CEST Spectrum:** A series of 1D NMR spectra were acquired using a 3 second saturation pulse applied at 20.97 μT power and with saturation frequencies spanning +35 to -35 ppm in 0.25 ppm increments (NEX=4, 37.3°C, 11.7T). Water peak heights were used to construct CEST spectra of a sample before and after adding the enzyme. MTRasym analysis was performed to measure the magnitude of CEST from the reactants and the Tm-DO3A-cadaverine-ZQG product (Fig. 2).

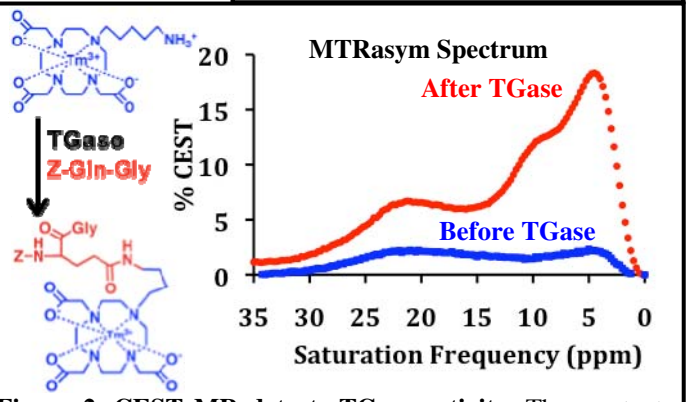

Figure 2. CEST MR detects TGase activity. The reactants show a broad, nonspecific MTRasym baseline with 1.5-2% CEST between 3 and 25 ppm. The product after the TGase reaction showed three specific peaks, which is consistent with three amides in the product and at least two amides that experience a pseudocontact shift from Tm(III).

RESULTS: The synthesis intermediates and final product prior to chelation were confirmed with Mass Spectrometry and NMR spectroscopy. HPLC of the reactants showed retention times of 6.6-6.9 min for Tm-DO3A-cadaverine and 24.6 min for ZQG, and HPLC of the products showed the same peaks plus a new peak at 10.6 min. The mixture of Tm-DO3A-cadaverine, ZQG, and glutathione reactants showed a broad, nonspecific MTRasym baseline with 1.5-2.0% CEST between 3 and 25 ppm (Fig. 2). The product after the TGase reaction showed three specific peaks in the MTRasym spectrum, with 6.7% CEST at 21.25 ppm, 12.2% CEST at 9.5 ppm, and 18.3% CEST at 4.5 ppm. The magnitudes of the three CEST effects of the products relative to the MTRasym "baseline" of the reactants validated that each CEST effect had >99% probability of being real.

DISCUSSION: The CEST effects at 21.25 and 9.5 ppm from the product must arise from a pseudocontact shift caused by close proximity of Tm(III) to an amide, which validates that Tm-DO3A-cadaverine can detect TGase enzyme activity. The appearance of three CEST effects after the TGase reaction is consistent with the three amides that are in close proximity to Tm(III) in the product, which provides supporting evidence for forming the product. The weak, broad MTRasym baseline of the reactants may possibly indicate that ZQG and glutathione non-covalently associate with Tm-DO3A-cadaverine prior to the reaction, and glutathione may possibly non-covalently interact with the product to form a strong CEST effect, which will be evaluated in subsequent studies.

REFERENCES: **1.** Kapil M, et al. *Biochem Pharmacol* 2010;80:1921-1929. **2.** Tei L, et al. *Contrast Media Molec Imaging* 2011;5:213-222. **3.** Yoo B, et al. *J Am Chem Soc* 2006;128:14032-14033. **4.** Chauvin T, et al. Angewandte Chemie 2008;47:4370-4372. **5.** Yoo B, et al. *Tet Lett* 2009;50:4459-4462. **6.** Suchy M, et al. *Org Biomol Chem* 2010;8:2560-2566. **7.** Li Y, et al. *Contrast Media Molec Imaging* 2011;6:219-228. **8.** Liu G, *J Am Chem Soc* 2011; *in press*.