Large-shift, Rapid Exchange Endogenous CEST Contrast for Reporter Gene Product Design

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INTRODUCTION: Monitoring the spread and persistence of cell- and viral-based therapeutics, such as oncolytic virotherapy (**OV**) and CAR-T cell immunotherapy, is essential to unlocking their potential and revolutionizing the treatment of many diseases. We have previously optimized the MRI-sensitive chemical exchange saturation transfer (**CEST**) contrast at 3.5 ppm of a reporter gene protein product[1–4] that can be produced by cells and therefore detectable without injection of another agent. However, larger chemical shifts and higher exchange rates would improve selectivity and specificity. Here we present peptide and protein CEST contrast at >4 ppm which is more easily distinguished from the endogenous amide signal.

METHODS: 4-5 mg of peptide (GenScript) or 34.5 mg of glucoamylase protein (Sigma) were resuspended in 600 μL of 1x phosphate-buffered saline and titrated to pH 7.3 at room temperature. ^{1}H NMR was performed at 37 °C on a 14 T scanner. Ultrafast z-spectroscopy[5] employed saturation powers ranging from 1-5 μT. Using custom MATLAB scripts with scripts written by Moritz Zaiss' group, Z-spectral peaks were fit to Pseudo-Voigt lineshapes and exchange rates were quantified by the Quantification of Exchange *via* Saturation Power (**QUESP**)[6,7] method using the fitted amplitudes and the measured water T_1 time constant. Peptide structure predictions were performed using PEP-FOLD4 online software[8].

RESULTS: We discovered a tryptophan (**Trp**)-containing peptide which showed a large resonance at 4.2 ppm (**Fig. 1A**). Peptide structure simulation (**Fig. 1B**) and CEST z-spectroscopy with various amino acid substitutions (data not shown) revealed that the 4.2 ppm CEST peak arises from a cation-π interaction[9] between a lysine (**Lys**) sidechain amine and two Trp indole rings. We measured the 4.2 ppm proton exchange rate to be 1930 s^{-1} (data not shown). We found that the protein glucoamylase has two highly-shifted, CEST-detectable proton pools at 7.3 and 9.8 ppm (**Fig. 2A**) due to a unique cation-π interacting complex[9] (**Fig. 2B**), in agreement with a previous conventional NMR study[10]. The larger CEST pool at 7.3 ppm exchanged with a rate constant of 2470 s^{-1} (data not shown).

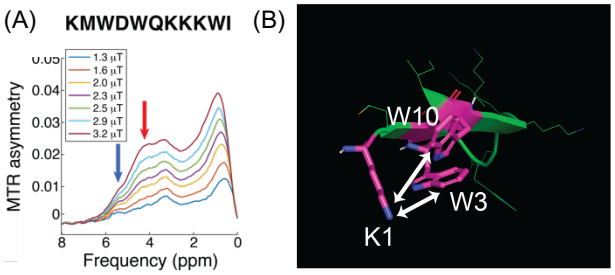

Figure 1. (A) MTR asymmetry of Trp peptide containing the novel 4.2 ppm contrast (red arrow) and Trp indole NH contrast at 5.3 ppm (blue arrow). (B) Structure prediction of the same peptide showing a cation-π interaction between the Lys (K1) and Trp (W3, W10) sidechains.

DISCUSSION: Endogenous Trp residues give CEST contrast at 5.3 ppm downfield from water[11], but the contrast is relatively weak (**Fig. 1A**, blue arrow). We show that interactions between aromatic amino acid sidechains and other groups generate large, highly shifted CEST peaks. Importantly, the glucoamylase CEST signals we found are robust at physiological temperature and pH.

CONCLUSION: Our discovery of highly-shifted CEST contrast that can be engineered into a native protein product provides a powerful tool for selectively and sensitively monitoring the spread and activation status of cell- and viral-based therapies. In future work, we will develop a stable reporter gene product and test it with *in vivo* MRI to evaluate OV therapeutic efficacy.

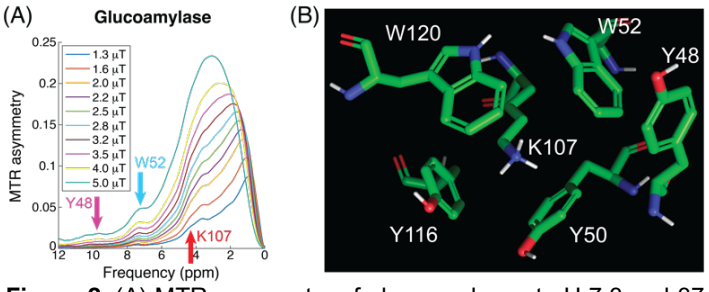

Figure 2. (A) MTR asymmetry of glucoamylase at pH 7.3 and 37 °C. Arrow labels indicate the putative residue assignments[10]. (B) Cation-π complex causing the highly-shifted CEST proton pools.

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