Enhanced 129Xe Hyper-CEST Efficiency Using PK11195 Functionalized Cryptophane-A

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INTRODUCTION: Hyperpolarized (HP) ^{129}Xe gas as an MRI probe has become a highly attractive field of study for MR researchers, as it provides a 100,000-fold signal enhancement compared to thermally polarized ^{129}Xe, is extremely sensitive to its molecular environment and has a large chemical shift range of over 200 ppm. ^{129}Xe is also soluble in a wide variety of biological media including blood, allowing for *in vivo* molecular imaging. Cryptophane-A molecules can encapsulate ^{129}Xe, causing a shift in the ^{129}Xe NMR spectrum of approximately +65 ppm with respect to the gas phase[1]. Functionalized cryptophane-A molecules can potentially dissolve into the blood stream and are carried to biological targets of interest where they then encapsulate dissolved ^{129}Xe. Here, the encapsulated ^{129}Xe is in exchange with the dissolved phase ^{129}Xe so that **C**hemical **E**xchange **S**aturation **T**ransfer (CEST) can be used to achieve further signal enhancement. As a result, a 10^8-fold signal enhancement is possible with the combination of HP ^{129}Xe and CEST, a technique termed Hyper-CEST. Hyper-CEST can be used to detect nanomolar concentrations of biomarkers[2], such as proteins associated with cancers. In this study, a more efficient pulse sequence was used to demonstrate Hyper-CEST, which provided higher Hyper-CEST efficiency and a much greater signal enhancement than has previously been achieved. The cryptophanes used for Hyper-CEST in this study have been functionalized with PK11195; and when bound to ^{129}Xe, cause a shift in the ^{129}Xe NMR spectrum of approximately +78 ppm. PK11195 targets peripheral benzodiazapine receptors (PBRs) present in glial cells at inflammation sites in the body; and thus, our method of Hyper-CEST has the potential to detect these inflammation sites caused by diseases such as COPD or arthritis.

METHODS: 2.5 mL of 30 mM PK11195 functionalized cryptophane-A dissolved in DMSO was shaken with 2.5 mL of HP ^{129}Xe in a plastic 5 mL LuerLok syringe prior to ^{129}Xe NMR spectroscopy. HP xenon isotopically enriched to 83.5% ^{129}Xe was prepared with a Xemed xenon polarizer, yielding up to 10% polarization. Spectroscopy of the sample was performed on a 3T whole-body Philips Achieva MRI using a T/R quadrature birdcage coil tuned to 35.33 MHz. Spectra were acquired after presaturation pulses were applied at +78 ppm with respect to the gas phase (on the cryptophane-encapsulated ^{129}Xe resonance). The presaturation pulses consisted of 6 ms duration, 3-lobe sinc pulses, applied with 3 ms inter-pulse spacing, followed by a crusher gradient. Four sets of pulses were used consisting of 3, 5, 7 and 10 pulses. Hyper-CEST imaging was also performed using a gradient echo pulse sequence preceded by a train of 10 presaturation pulses. Data were processed using Matlab and MNova NMR processing software.

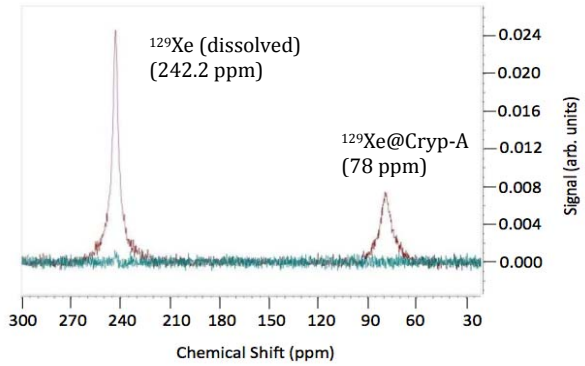

Figure 1: ^{129}Xe Hyper-CEST NMR spectrum of PK11195 functionalized cryptophane-A with presaturation pulses on (blue) and off (red).

RESULTS AND DISCUSSION: Figure 1 shows the MR spectrum of a 5.0 mL syringe containing HP ^{129}Xe gas dissolved in cryptophane-A solution (red). Two separate resonances can be seen at +78 and +242.2 ppm with respect to the gas phase (not shown), which can be attributed to ^{129}Xe encapsulated by cryptophane-A and ^{129}Xe dissolved in DMSO, respectively. Overlaid on this spectrum is a separate MR spectrum (blue) of the same sample acquired after applying 10 presaturation pulses at the frequency of encapsulated ^{129}Xe. By using pulsed saturation to demonstrate Hyper-CEST as opposed to continuous wave saturation, we were able to limit the specific absorption rate (SAR). The SNRs of the dissolved and encapsulated ^{129}Xe peaks were calculated to be 94.3 and 40.3, respectively, for the spectrum acquired without presaturation pulses. The dissolved ^{129}Xe peak SNR was decreased by 87% to 12.3 after applying 10 presaturation pulses at the encapsulated ^{129}Xe resonance. This signal decrease, which can be attributed to Hyper-CEST, is to our knowledge the largest decrease yet reported. To verify that this signal decrease was actually due to Hyper-CEST, we applied presaturation pulses at +406.4 ppm, on the opposite side of the dissolved phase signal, and observed no decrease in the dissolved phase signal. Figure 2 is a plot of percent decrease in SNR of the dissolved ^{129}Xe peak after Hyper-CEST vs. the number of presaturation pulses, suggesting that Hyper-CEST effect increases linearly with increasing number of presaturation pulses. Figure 3a is a ^{129}Xe dissolved phase image after presaturation pulses at +164.2 ppm, which served as a control image. Figure 3b is the ^{129}Xe dissolved phase image after applying presaturation pulses at -164.2 ppm, showing depletion in dissolved phase ^{129}Xe signal due to Hyper-CEST. Figure 3c is a subtraction image (a-b), which spatially locates the PK11195-cryptophane-A molecules in solution. By providing signal enhancement of several orders of magnitude compared to conventional MRI, Hyper-CEST has potential to serve as a promising molecular imaging technique. Since the cryptophane molecules are functionalized with PK11195, which targets PBRs, this technique can potentially detect inflammation sites in the body, such as in the case of COPD and arthritis.

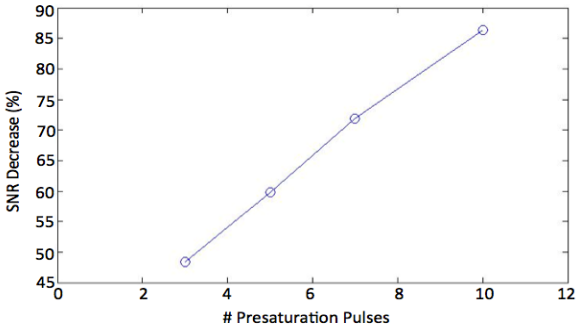

Figure 2: Plot of percent SNR decrease vs. number of presaturation pulses applied at the cryptophane-A resonance.

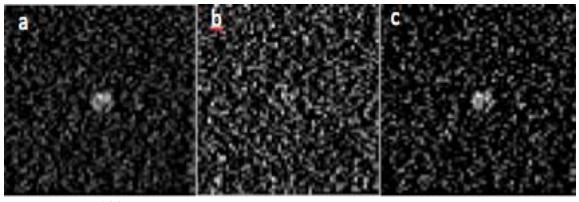

Figure 3: ^{129}Xe dissolved phase images after presaturation pulses at +164.2 ppm (control) (a); and presaturation pulses at -164.2 ppm (Hyper-CEST) (b); and a subtraction image (a-b) spatially locating the PK11195-cryptophane-A (c).

CONCLUSION: Hyper-CEST holds promise for molecular imaging using MRI. This work demonstrated achievement of enhanced Hyper-CEST efficiency with signal depletion approaching 90%, as well as demonstrating imaging using Hyper-CEST to achieve high contrast for future use in molecular imaging.

REFERENCES: 1. Leif Schröder et al. (2006) Science 314:446-449. 2. Todd Stevens et al. (2012) Magn Reson Med. 69(5): 1245-52.