Comparison of CEST Analysis in Z and 1/Z for Exchange Rate Quantification in Tissue-Like Systems

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Background: Estimating exchange rates from *in vivo* imaging data is complicated by the presence of multiple overlapping CEST pools, CEST effect dilution by the large semisolid proton pool, and concomitant factors such as B_0 inhomogeneity. As such, it is desirable to develop a method for rapid and accurate quantification of exchange rates in tissue-like systems, such as cross-linked bovine serum albumin (BSA), using NMR spectroscopy. Fitting and summing CEST contrasts in the *1/Z* domain, rather than in the *Z* domain as is typically done, aligns better with established CEST theory and produces meaningfully different quantitative results in the presence of magnetization transfer (MT) or spillover due to direct water saturation.

Here, we demonstrate the utility of ultrafast Z-spectroscopy (UFZS) (2,3) combined with Pseudo-Voigt fitting (4) and *1/Z* analysis for rapid and accurate exchange rate quantification in tissue-like systems with overlapping CEST pools and strong MT effects.

Methods: Tissue-like samples consisting of 25mM creatine in phosphate-buffered saline (PBS) with 5-10% heat-crosslinked BSA were prepared in 5 mm NMR tubes. First, the longitudinal relaxation rate of water (R_{1a}) in the sample was measured using a standard inversion recovery pulse sequence with 16 inversion times, ranging from 2 ms to 30 s. Then, 12 individual Z-spectra were acquired from -15 to 15 ppm with saturation amplitudes ranging from 0 to 3.8 μT using a gradient-encoded UFZS sequence. All experiments were performed at 37.0°C. To isolate CEST pools, the MT contribution was first fitted with a Super-Lorentzian lineshape using far-off-resonance points in the Z-spectrum (±8 to 15 ppm). Subsequently, hydroxyl and guanidyl peaks were fit in the 1/*Z* domain using an iterative least-squares approach. Finally, exchange rates were quantified using the analytical "QUantification of Exchange rates using varying Saturation Powers" (QUESP) equations (5).

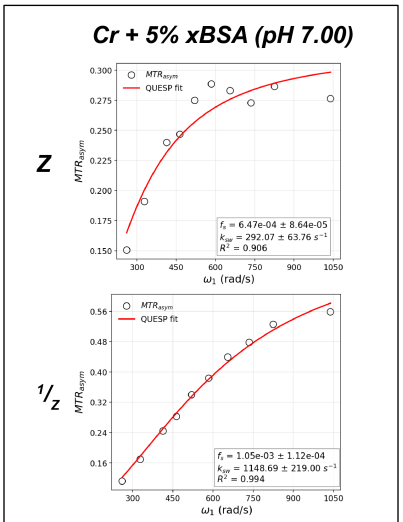

Figure 1. Representative QUESP fitting results for 25mM creatine in 5% heat-crosslinked BSA.

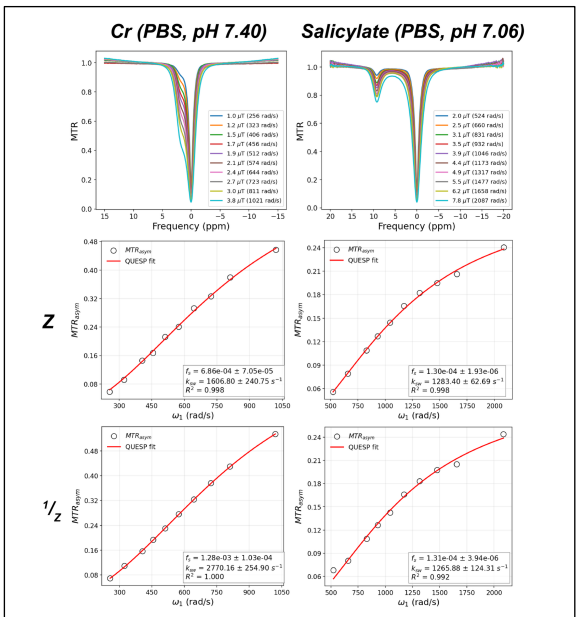

Figure 2. Representative Z-spectra and QUESP fitting results for 25mM creatine and 18mM salicylic acid in PBS.

Results: For creatine in 5% heat-crosslinked BSA, fitting in *1/Z* resulted in a 4-fold higher quantified exchange rate (k_{sw}) and a 60% larger proton volume fraction (f_s) compared to fitting in *Z* (Figure 1). When quantified in the *1/Z* domain, creatine guanidyl exchange rates agreed within margin of error regardless of semisolid content (k_{sw}=1149±219 s^{-1} vs. k_{sw}=1042±302 s^{-1} in 5% vs. 10% heat-crosslinked BSA respectively, both pH 7.00).

Even in systems without semisolid magnetization transfer (e.g., creatine in PBS, Figure 2), spectral overlap between the water and exchangeable proton peaks affected the quantified exchange rates depending on the fitting method. Fitting in *1/Z* provided quantified exchange rates that are more consistent with previously reported results (6). The guanidyl proton f_s determined from the 1/Z analysis was slightly overestimated compared to the ground truth for Cr in 5% BSA (f_s=1.05x10^{-3}±0.11x10^{-3}, $f_{s,ground\ truth}$=0.91x10^{-3}). In contrast, the f_s was significantly underestimated (f_s=0.65x10^{-3}±0.86x10^{-3}) with the Z analysis. In general, estimated proton volume fractions were more accurate when fit in *1/Z* than *Z*. Alternatively, for systems with minimal spectral overlap (e.g., salicylate at 9.2 ppm in PBS), quantified exchange rates and proton volume fractions agreed within margin of error regardless of the domain used for fitting (Figure 2).

Conclusion: Accurate estimations of *in vivo* exchange rates are crucial for the development of quantitative methods utilizing CEST, such as pH imaging and CEST magnetic resonance fingerprinting. Here, we demonstrate that quantified exchange rates can diverge significantly based on whether CEST peaks are fit in *Z* vs. *1/Z*. This difference is particularly apparent in tissue-like systems with strong MT and spillover effects.

References: **1.** Zaiss M, Bachert P. *Phys Med Biol*. 2013;58(22):R221-R269. **2.** Boutin C, et al. *J Phys Chem Lett*. 2013;4(23):4172-4176. **3.** Xu X, et al. *Angew Chem Int Ed*. 2013;52(32):8281-8284. **4.** Zhang L, et al. *Quant Imaging Med Surg*. 2019;9(10):1714-1730. **5.** Zaiss M, et al. *Magn Reson Med*. 2018;79(3):1708-1721. **6.** Goerke S, et al. *NMR Biomed*. 2014;27(5):507-518.