

Imaging Ca²⁺ using Fluorine Chemical Exchange Saturation Transfer (¹⁹F-CEST)

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Target Audience: Researchers and clinicians who are interested in molecular and cellular imaging, those developing new methods for CEST-MRI, and those developing new contrast agents for ¹⁹F MRI.

Purpose: To develop a chemical exchange saturation transfer (CEST) approach for specific imaging of free *endogenous* metal ions using the binding kinetics of these ions through the chemical exchange with fluorinated substrates, i.e. ¹⁹F-CEST.

Methods: ¹⁹F-CEST experiments were performed on solutions containing 1,2-Bis-[2-bis(carboxymethyl)amino-5-fluorophenoxy]ethane (5FBAPTA), with and without divalent cations (M²⁺). **Spectroscopy:** Experiments were performed on an 11.7T NMR spectrometer (Bruker). 5FBAPTA (AnaSpec, Inc.) was dissolved in 40 mM HEPES buffer (pH=7.0) to a concentration of 5 mM, and CaCl₂ was dissolved to a final Ca²⁺ concentration of 50 μM. ¹⁹F-CEST spectra (z-spectra) were acquired with a saturation transfer sequence consisting of a saturation pulse (B₁=4.7 μT, 4 sec) with variable offset (from -7.5 to +7.5 ppm relative to the 5FBAPTA frequency set at 0 ppm). Experiments were performed without (room temperature, RT) and with sample heating (37°C). The MTR_{asym}=100×(S_{Δω} - S_{+Δω})/S₀ was computed at different offsets Δω, where S₀ is the 5FBAPTA signal without saturation. **MRI:** Imaging experiments were performed on a 16.4T MRI scanner (Bruker). 5FBAPTA was dissolved to 10 mM in 40mM HEPES, pH=7.0, with or without 100 μM of M²⁺ (Mg²⁺, Zn²⁺, or Ca²⁺). ¹⁹F-CEST images were acquired using a continuous wave presaturation pulse (B₁=3.6 μT, 3sec) followed by a multi-echo MRI pulse sequence (RARE, rare factor 4, TR/TE=6000/10 ms). FOV of 4×4 cm, matrix 32×32 and slice thickness 10mm. Mean ¹⁹F-CEST spectra were derived after B₀ correction for each voxel using MatLab. MTR_{asym} plots and maps were calculated as described above.

Results: Fig. 1a illustrates the chemical shift offsets (Δω) of the 5FBAPTA at the ¹⁹F NMR frequencies upon complexation with various divalent cations (M²⁺). Fig. 1b depicts the dynamic exchange process between the free 5FBAPTA and Ca²⁺-bound 5FBAPTA, i.e., [Ca-5FBAPTA], allowing indirect detection of low Ca²⁺ concentrations by using saturation transfer (Fig. 1c). Fig. 1c shows the ¹⁹F-CEST-spectra and MTR_{asym} plots of 5 mM 5FBAPTA in the presence of 50 μM Ca²⁺ in HEPES buffer (pH=7.0). The dynamic ion exchange process between 5FBAPTA and [Ca-5FBAPTA], which increases with temperature, results in an observed increase in the MTR_{asym} value at Δω=5.8 ppm (Δω of [Ca-5FBAPTA]) resulting in a change in the 5FBAPTA signal.

The ¹⁹F-CEST MR images shown in Fig. 2 clearly demonstrate the specificity of our approach. On conventional ¹⁹F MRI (Fig. 2a) no difference in contrast could be observed for the different tubes (no M²⁺, Mg²⁺, Zn²⁺, and Ca²⁺) as this detects only the free 5FBAPTA. On ¹⁹F-CEST MRI however, only the Ca²⁺ containing solution generated contrast (Fig. 2b). Here, the saturation pulse was applied at Δω=5.8 ppm from the 5FBAPTA resonance (0 ppm). Fig. 2c shows the mean ¹⁹F-CEST MTR_{asym} plot for the tube containing 5FBAPTA in the presence of Ca²⁺.

Discussion: The variance in the Δω of [M²⁺-5FBAPTA] complex for each examined M²⁺ makes ¹⁹F-CEST MRI more specific than relaxation based ¹H MRI methodologies. Its specificity is probably comparable to that of PARACEST agents used for Ca²⁺ and Zn²⁺ binding^{2,3}, but no paramagnetic agent is needed. The higher saturation transfer effect for 5FBAPTA obtained at 37°C as compared to RT (Fig. 1c) is due to the faster ion exchange rate between 5FBAPTA and [Ca-5FBAPTA]. This confirms that the observed effect is due to the dynamic binding kinetics process, with our approach following the principles of ¹H-CEST MRI and this exchange mechanism can be classified as “molecular exchange”⁴. The specificity of the ¹⁹F-CEST method proposed here is not just due to the different Δω values of the examined ions (Fig. 1a), but also due to the different dissociation constants (K_d) between 5FBAPTA and the divalent metal complex [M²⁺-5FBAPTA], which determines the exchange rate (k_{ex}) and therefore the ¹⁹F-CEST contrast. The K_d for Ca²⁺ results in slow to intermediate k_{ex} on the NMR time scale⁵, while the K_d values for Mg²⁺ and Zn²⁺ result in k_{ex} values that are too fast and too slow, respectively¹, preventing the observation of signal changes in the ¹⁹F-CEST experiment.

Conclusion: We have developed a new approach for Ca²⁺ monitoring using MRI, in which the conventional amplification strategy and exchange sensitivity of CEST are combined with the Δω specificity of the ¹⁹F frequency in free and bound substrate to obtain MR images of calcium binding kinetics. Supported by MSCRFII-0161-00, R01EB015032 and MSCRFF-0103-00.

References: 1. G. A. Smith *et al.*, *Proc Natl Acad Sci U S A* **80**, 7178 (1983). 2. G. Angelovski *et al.*, *Bioorg Med Chem* **19**, 1097 (2011). 3. R. Trokowski *et al.*, *Angew Chem Int Ed Engl* **44**, 6920 (2005). 4. P. C. van Zijl, N. N. Yadav, *Magn Reson Med* **65**, 927 (2011). 5. H. Gilboa *et al.*, *NMR Biomed* **7**, 330 (1994)

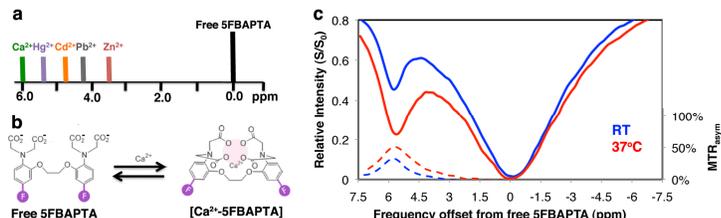


Fig 1. a) Schematic illustration of the chemical shift offsets (Δω) at the ¹⁹F NMR spectrum of 5FBAPTA upon chelation with different bivalent cations. **b)** Demonstration of the dynamic exchange process between free and Ca²⁺-bound 5FBAPTA. **c)** ¹⁹F-CEST spectra (solid lines) and MTR_{asym} plots (dashed lines) for the complex [Ca²⁺-5FBAPTA] at room temperature (RT, blue) and 37°C (red) obtained at 11.7T.

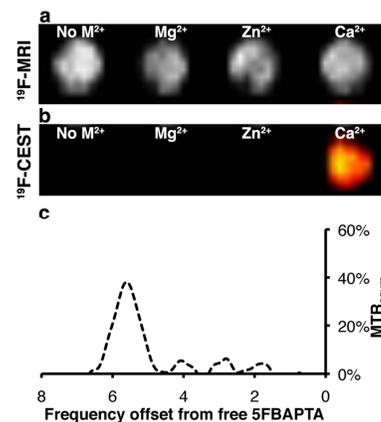


Fig 2. a) ¹⁹F MRI of tubes containing 10 mM of 5FBAPTA obtained at 16.4T. Tubes contained either no M²⁺ content or one of the ions Mg²⁺, Zn²⁺ or Ca²⁺ at 100 μM concentration. **b)** MTR_{asym} maps (Δω=5.8 ppm) obtained from the ¹⁹F-CEST experiment of the tubes shown in a. **c)** MTR_{asym} plots (dashed lines) of the Ca²⁺ containing tube.