

Magnetization Transfer Detection of GFP: A New MRI Gene Reporter

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Introduction: To visualize gene expression via any imaging modality, a reporter system needs to be instituted that causes sufficient contrast between the tissues where the gene is expressed and where it is not expressed. Green Fluorescent Protein (GFP) is a widely used molecular and gene expression marker. However, its use in *in vivo* imaging has been limited to transparent tissue due to the limitations of optical imaging. Here, we report a technique to detect GFP by using Magnetic Resonance Imaging (MRI). Magnetization Transfer Contrast (MTC), also mentioned as Chemical Exchange Saturation Transfer (CEST), a technique previously used to detect changes in other proteins like myelin and collagen is used to detect GFP both *in vitro* and *in vivo*. We show that the technique produces values that are protein specific, and we have preliminary indications that it is concentration dependent. This provides a flexible, non-invasive *in vivo* molecular imaging system exclusively dependent on the concentration of the reporter GFP.

Methods: Animals: We procured three different strains of GFP expressing mice. Actin-GFP mice which express eGFP ubiquitously under the control of the β -actin promoter, GIN Mice, which express eGFP under the control of the *Gad1* promoter in GABAergic neurons, and L7-GFP mice, which express eGFP under the control of the L7 promoter in purkinje cells in the cerebellum. Corresponding age matched controls were imaged. All the animals used in this study were handled in compliance with institutional and national regulations and policies. The protocols were approved by the Institutional Animal Subjects Committee at Baylor College of Medicine.

Imaging Protocol: Animals were anesthetized by isoflurane gas at 5% in oxygen and placed into a mouse holder where they were kept under anesthesia at a nominal 2% isoflurane in oxygen. Imaging was done utilizing a Bruker Avance Biospec, 9.4 T spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. During imaging the animal body temperature was maintained at 37.0°C using an animal heating system (SA Instruments, Stony Brook, NY). T1 weighted images were taken before MTC to locate ideal MTC slice placement. MTC imaging pulse sequence comprised a pre-saturation square pulse at the designated offset frequency followed by a spin echo sequence with TE/TR=10.25/1460 msec. Images were recorded with a 256x256 matrix, Field of View = 3x3 cm, slice thickness = 2mm, and average = 1. Pre-saturation off-resonance pulses ranged from 0 to 20 kHz.

Data Analysis: Magnetization Transfer Ratios (MTR) in the form of $MTR = (Unsaturated - Saturated) / Unsaturated$ were calculated from the signal intensities of regions of interest (ROI) using Paravision software (Bruker Biospin, Billerica, MA). Graphs and statistical analyses were conducted with Prism (GraphPad Software, San Diego, CA). Pixel by pixel MTR calculations were performed in Amira 3D Visualization software to generate pseudo-colored images. Data is shown as Mean \pm SD. Comparison at each offset frequency was performed using Student's t tests with significance at $P < 0.05$.

Results: In Vitro Phantoms: Initial tests compared MTC profiles of eGFP and Bovine Serum Albumin (BSA). Both proteins were at equal concentrations of 0.1 mg/ml in 1 ml vials. BSA was chosen as a non-specific control to compare against GFP. The goal was to find the frequency at which there is the largest difference between GFP and BSA control. Both phantoms were imaged first without and then with MTC. 5 MTC datasets at 0.5, 0.7, 1, 5, and 10 kHz were acquired. From the images, the MTR was calculated and is shown in Figure 1B. The largest differences are at 0.5, 0.7, and 1 kHz and each was significant by t test with a P-value < 0.01 . Figure 1A also shows a pseudocolored pixel by pixel MTR calculation that visually shows a clear difference. Further analysis was done with a 1:3 dilution of the previous GFP solution imaged with the same parameters. The MTR profile of the diluted GFP was in between the GFP and BSA curves in Figure 1B suggesting a dose dependent effect. (Data not shown)

GFP Mice: Mice Region based MTR calculations for the Actin model are shown in Figure 1D for the different offset frequencies. (Data for the other models not shown.) In all three models a significant difference was seen between GFP and control animals at 1 kHz. Other frequencies show significant differences in some animals but not all. Figure 1C also shows sample pixel by pixel calculations for the Actin model. (Data for the other models not shown.) MTR is not uniform but there is still a clearly discernable difference between GFP producing and control animals.

Discussion: There are at least 4 MRI based reporters in the literature: ferritin¹, transferrin², tyrosinase³, and a β -galactosidase activated gadolinium chelate⁴. All these alternatives require both a reporter and a substrate so measurements are dependent on the concentrations of both. Additionally, the substrate is potentially toxic in all of these systems. There is also a similar CEST reporter system based on an artificial lysine rich-protein⁵. GFP is already a widely used protein marker. Our data suggest that GFP could be a useful alternative to track gene expression *in vivo* providing a flexible, non-invasive *in vivo* MRI molecular imaging system exclusively dependent on the concentration of the reporter GFP with negligible toxicity issues.

References: 1. Cohen, B. et al. *Nat Med* **13**, 498-503(2007). 2. Moore, A. et al. *Radiology* **221**, 244-50(2001). 3. Enochs, W.S. et al. *Radiology* **204**, 417-23(1997). 4. Louie, A.Y. et al. *Nat Biotech* **18**, 321-325(2000). 5. Gilad, A.A. et al. *Nat Biotechnol* **25**, 217-9(2007).

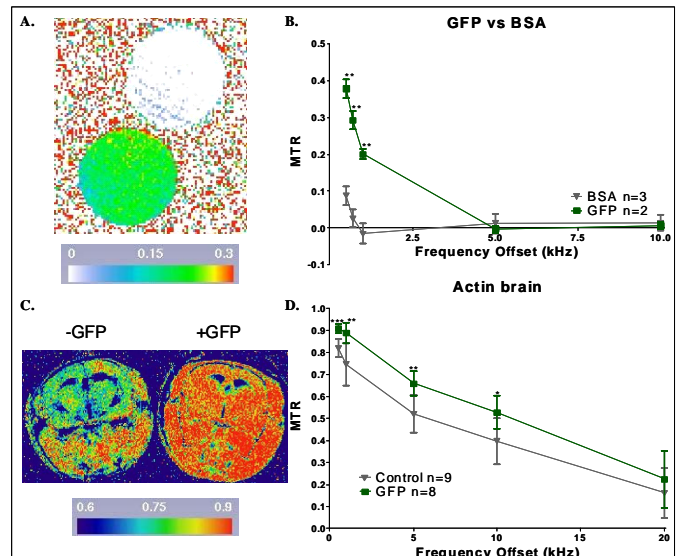


Figure 1. A. Pseudocolored pixel by pixel MTR calculation for 1 kHz offset for GFP vs. BSA comparison. B. Region based MTR calculations for the different frequency offsets for GFP vs. BSA Protein comparison. P-values < 0.01 for 0.5, 1, and 5 kHz. C. Pseudocolored pixel by pixel MTR calculation for 1 kHz offset for Actin GFP model. D. Region based MTR calculations for the different frequency offsets for Actin GFP model. P-values < 0.05 for 10 kHz, < 0.01 for 1 and 5 kHz, and < 0.001 for 0.5.