Supercharged Green Fluorescent Proteins as Bimodal Reporter Genes for CEST and Optical Imaging

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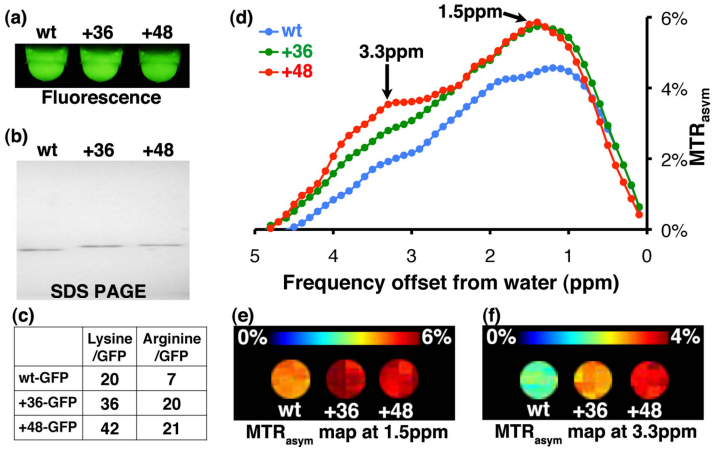
Target Audience: Researchers and clinicians who are interested in molecular and cellular MRI especially in non-invasive monitoring of gene expression and gene therapy.

Purpose: The overall goal is to modify the optical reporter gene green fluorescent protein (GFP) into an MRI-based reporter gene based on previous studies on the lysine rich protein (LRP)[1] and GFP[2].

Methods: *E. coli* optimized genes encoding to wild type GFP (wt) and its superpositively charged variants (+36 and +48), achieved by modifying the solvent-exposed amino acids to lysine or arginine[3], were obtained from Dr. David R. Liu (Harvard University, Cambridge, MA[3-5]). The proteins were expressed in BL21 (DE3) *E. coli* after induction in Magic Media™ and purified using cobalt-based immobilized metal affinity chromatography. The expression and purity was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Pure proteins were dialyzed against PBS and their CEST-MRI characteristics were measured using a modified RARE sequence (TR/TE=6000/9.4 ms) including a magnetization transfer module (B_1=4.7 μT/4000 ms). The absolute water resonant frequency shift was measured using a modified WASSR method, with the same parameters as in CEST imaging except TR=1.5 sec and B_1 saturation pulse=0.5 μT/250 ms. Mean CEST spectra were derived from a ROI for each sample after B_0 correction for each voxel using MatLab. MTR asymmetry (MTR_{asym})=100×($S_{-\Delta\omega}$ – $S_{+\Delta\omega}$)/ S_0 was computed at different offsets of Δω.



(c)

	Lysine /GFP	Arginine /GFP
wt-GFP	20	7
+36-GFP	36	20
+48-GFP	42	21

Fig 1. Characterization of wt-GFP and its superpositive mutants +36-GFP and +48-GFP. a) Fluorescence. b) SDS-PAGE of purified GFPs. c) Number of lysine and arginine amino acids in each GFP (total 239 amino acids). d) MTR_{asym} plots. e) MTR_{asym} maps (Δω=1.5 ppm). e) MTR_{asym} maps (Δω=3.3 ppm).

Results: Fig. 1a shows fluorescence images of solutions containing the wt, +36, and +48 GFP variants. The high purity of the three proteins was validated by SDS-PAGE (Fig. 1b). The CEST MRI characteristics of the three examined GFPs (wt, +36, and +48) showed that both superpositive GFPs (+36 and +48) generated higher MTR_{asym} values as compared to wt-GFP at both 1.5 ppm (guanidine protons of arginine) and 3.3 ppm (amide protons) (Fig. 1d-f). The +48 GFP generated higher MTR_{asym} values at the amide frequencies (3.3 ppm) than the +36 mutant. Fig. 1e-f display MTR_{asym} maps that reveal the correlation between the protein charge and the CEST contrast.

Discussion: Superpositively charged GFPs are highly resistant to aggregation and retain high fluorescence levels even after being boiled and cooled[3]. Here we show that the +36 and +48 GFPs mutants generate high CEST MRI contrast. Both examined mutants contain an equal number of arginine residues (20 and 21, respectively) and therefore generated the same MTR_{asym} value from the guanidine exchangeable protons at Δω=1.5 ppm. In contrast, as +48-GFP mutant has more lysines as compared to +36-GFP (42 and 36, respectively), it generated higher MTR_{asym} values at the resonance of the amide protons at Δω=3.3 ppm.

Conclusion: Superpositively charged GFP, a lysine/arginine rich fluorescent protein, is an excellent reporter gene for CEST MRI while retaining its capability as an optical reporter gene. Supported by MSCRFF-0103-00.

References: 1. A. A. Gilad *et al.*, *Nat Biotechnol* **25**, 217 (2007). 2. C. J. Perez-Torres, C. A. Massaad, S. G. Hilsenbeck, F. Serrano, R. G. Pautler, *Neuroimage* **50**, 375 (2010). 3. M. S. Lawrence, K. J. Phillips, D. R. Liu, *J Am Chem Soc* **129**, 10110 (2007). 4. J. J. Cronican *et al.*, *ACS Chem Biol* **5**, 747 (2010). 5. B. R. McNaughton, J. J. Cronican, D. B. Thompson, D. R. Liu, *Proc Natl Acad Sci U S A* **106**, 6111 (2009).