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

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Experimental Procedures

Protein expression and purification: *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, were obtained from Dr. David R. Liu (Harvard University, Cambridge, MA)[1]. 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 2 M NaCl in PBS.

CEST MRI: CEST-MRI experiments were performed on a vertical bore 11.7 T Bruker Avance system, with the sample temperature controlled to be 37^{o}C along the whole experiment. One mm capillaries were loaded with protein solutions in duplicate and located in the middle of a 20 mm rf bird cage coil. A slice thickness of 1 mm, a FOV = 17×16 mm^2, and a matrix size = 128×64,resulted in a resolution of 0.133×0.25 mm^2 for each CEST/WASSR experiment. CEST-MRI characteristics were measured using a modified RARE sequence (TR/TE=6000/9.4 ms), including a magnetization transfer module (B_1=4000 ms and variable powers of 2.4 μT, 3.6 μT, 4.7 μT, and 7.2 μT) from -5 to +5 ppm, in increments of 0.2 ppm. To correct for B_0 inhomogeneity, the absolute water resonance frequency shift was determined at each voxel using a modified WASSR method[2], 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 an ROI for each sample after B0 correction using MatLab. MTR asymmetry (MTR_{asym})=100×($S^{-\Delta\omega} - S^{+\Delta\omega}$)/$S^0$ was computed at different offsets of Δω.

Cloning: The synthetic genes encoded for wt, +36, and +48 GFP were optimized for expression in a mammalian setup and were purchased from Genscript (Piscataway, NJ). All three genes were sub-cloned into the pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA). The optimized genes sequences are as follows:

wt-GFP:
ATGGGACATCATCATCATCATCACGGCGGGGCTTCAAAAGGGGAGGAACTGTTTACTGGGGT
GGTGCCTATTCTGGTGGAGCTGGATGGAGACGTGAATGGCCACAAATTCTCTGTCCGAGGAGA
GGGAGAAGGAGATGCAACCAACGGAAAGCTGACACTGAAATTTATCTGCACCACAGGCAAGC
TGCCCGTGCCTTGGCCAACACTGGTCACTACCCTGACTTACGGCGTGCAGTGTTTCAGCCGAT
ATCCAGACCACATGAAGCAGCATGATTTCTTTAAAAGTGCTATGCCCGAGGGCTACGTGCAGG
AACGGACTATTTCATTCAAGGACGATGGGACATATAAAACTAGAGCAGAGGTGAAGTTTGAA
GGGGACACACTGGTCAACAGGATCGAGCTGAAGGGAATTGACTTCAAAGAAGATGGGAATAT
CCTGGGACACAAACTGGAGTACAACTTCAACAGCCATAACGTGTACATCACCGCAGATAAGC
AGAAAAACGGCATCAAGGCCAATTTCAAAATTAGACACAATGTGGAAGACGGCTCCGTCCAG
CTGGCCGATCATTACCAGCAGAACACCCCTATCGGAGACGGACCAGTGCTGCTGCCTGATAAT
CATTATCTGAGCACACAGTCCGCTCTGTCTAAGGACCCCAACGAGAAACGGGATCACATGGTG
CTGCTGGAATTTGTCACTGCCGCTGGCATTACCCACGGCATGGACGAGCTGTACAAG

+36-GFP:
ATGGGACATCATCATCATCATCACGGCGGAGCAAGCAAAGGCGAGAGGCTGTTTAGAGGGAA
AGTGCCAATCCTGGTGGAACTGAAAGGAGACGTGAACGGCCACAAGTTCTCTGTCCGAGGCA
AGGGGAAAGGAGATGCCACACGGGGAAAACTGACTCTGAAGTTTATCTGCACCACAGGCAAG
CTGCCCGTGCCTTGGCCAACCCTGGTCACTACCCTGACATACGGCGTGCAGTGTTTCAGCCGA
TATCCAAAACACATGAAGCGGCATGACTTCTTTAAAAGTGCTATGCCCAAGGGCTACGTGCAG
GAGCGCACCATTTCATTCAAGAAGGATGGGAAGTACAAGACCCGGGCCGAGGTGAAGTTTGA
AGGGAGGACCCTGGTCAACCGCATCAAGCTGAAAGGACGCGACTTCAAGGAAAAAGGGAAT
ATTCTGGGACACAAACTGAGATACAACTTCAACAGCCATAAGGTGTACATCACAGCAGATAA
GAGAAAAAACGGCATCAAGGCCAAATTCAAGATTAGGCACAATGTGAAGGACGGCTCCGTCC
AGCTGGCTGATCATTACCAGCAGAACACACCTATTGGCAGAGGGCCCGTGCTGCTGCCTAGGA
ATCATTATCTGAGCACTCGCTCCAAACTGTCTAAGGACCCCAAAGAGAAGCGGGATCACATG
GTGCTGCTGGAATTTGTCACCGCCGCTGGCATCAAACATGGGCGGGACGAGAGATACAAG

+48-GFP:
ATGGGGCATCATCATCATCATCACGGGGGCAGGTCCAAAGGAAAAAGACTGTTTAGAGGCAA
GGTCCCTATTCTGGTCAAGCTGAAAGGAGACGTGAACGGCCACAAGTTCTCTGTCCGAGGCAA
GGGGAAAGGAGATGCCACACGGGGAAAACTGACTCTGAAGTTTATCTGCACCACAGGCAAGC
TGCCCGTGCCTTGGCCAACCCTGGTCACTACCCTGACATACGGCGTGCAGTGTTTCAGCCGAT
ATCCAAAACACATGAAGCGGCATGACTTCTTTAAAAGTGCTATGCCCAAGGGCTACGTGCAG
GAGCGCACCATTTCATTCAAGAAGGATGGGAAGTACAAGACCCGGGCCGAAGTGAAGTTTAA
AGGGAGGACCCTGGTCAACCGCATCAAGCTGAAAGGACGCGACTTCAAGGAGAAAGGGAAT
ATTCTGGGACACAAACTGAGATACAACTTCAACAGCCATAAGGTGTACATCACAGCAGACAA
GAGAAAAAACGGCATCAAGGCCAAATTCAAGATTAGGCACAATGTGAAAGATGGCTCCGTCC
AGCTGGCTAAGCATTACCAGCAGAATACACCTATTGGCAGAGGGCCCGTGCTGCTGCCTAGG
AAGCATTATCTGAGCACTCGCTCCAAACTGTCTAAGGACCCCAAAGAGAAGCGGGATCACAT
GGTGCTGCTGGAATTTGTCACCGCCGCTGGCATCAAGCATGGGCGGGATAAAAGATACAAG

Expression in mammalian cells: Human Embryonic Kidney 293 cells (HEK-293T) were transfected with one of the following vectors, pcDNA3.1-wtGFP, pcDNA3.1-+36GFP, or pcDNA3.1-+48GFP, with the aid of the Lipofectamine-2000 (Invitrogen, Carlsbad, CA) transfection reagent. Twenty-four hours following transfection, a fluorescent microscope was used to monitor green fluorescence from transfected and non-transfected cells.

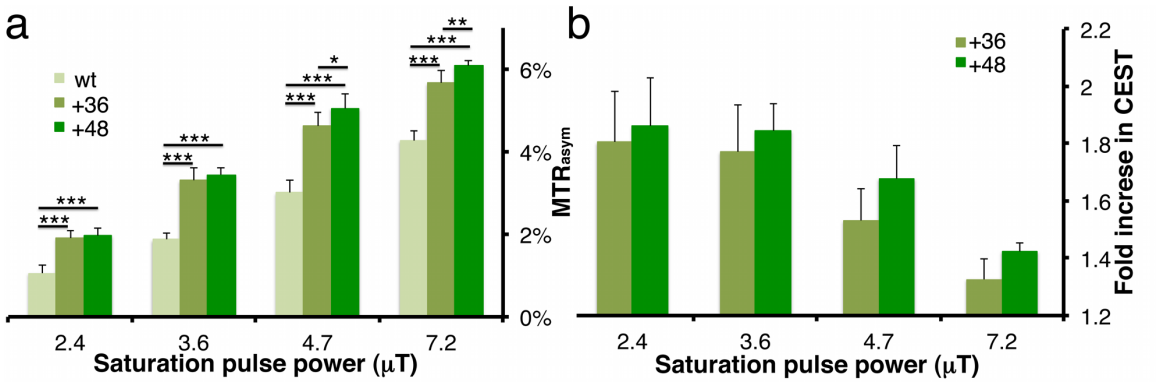

Figure S1. CEST characteristics of GFP proteins as obtained when a saturation pulse was applied at 1.8 ppm frequency offset. a) MTR_{asym} values at different rf power, and b) increase in the obtained MTR_{asym} value relative to wt GFP. CEST data of 1.25 mg/ mL pure protein solutions were acquired at 11.7 T, 37°C, pH=7.2, and B_1=4000 ms. N=7 for each sampled protein. P-values were calculated using a Student's t-test. * p<0.05, ** p<0.01, *** p<0.001.

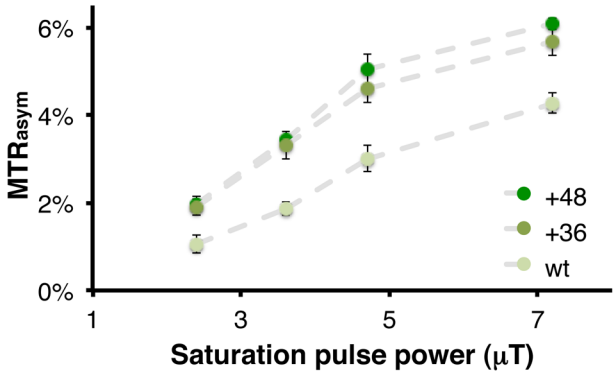

Figure S2. The dependency of the obtained MTR_{asym} values on the applied saturation pulse (B_1) power at Dw=1.8 ppm. Data of 1.25 mg/ mL pure protein solutions were acquired at 11.7 T, 37°C, pH=7.2, and B_1=4000 ms. N=7 for each sampled protein.

Table S1: Number of arginine residues and the measured MTR_{asym} value obtained at 1.8 ppm (1.25 mg/mL) at different B_1 powers.

	No. of arginines	MTR_{asym} B_1=2.4 mT	MTR_{asym} B_1=3.6 mT	MTR_{asym} B_1=4.7 mT	MTR_{asym} B_1=7.2 mT
wt GFP	7	1.1±0.2%	1.9±0.2%	3.0±0.3%	4.3±0.2%
+36 GFP	20	1.9±0.2%	3.3±0.3%	4.6±0.3%	5.7±0.3%
+48 GFP	21	2.0±0.2%	3.4±0.2%	5.1±0.3%	6.1±0.1%

References

1(a) M. S. Lawrence, K. J. Phillips and D. R. Liu, *J Am Chem Soc*, 2007, **129**, 10110; (b) J. J. Cronican, D. B. Thompson, K. T. Beier, B. R. McNaughton, C. L. Cepko and D. R. Liu, *ACS Chem Biol*, 2010, **5**, 747; (c) B. R. McNaughton, J. J. Cronican, D. B. Thompson and D. R. Liu, *Proc Natl Acad Sci USA*, 2009, **106**, 6111.

2) M. Kim, J. Gillen, B. A. Landman, J. Zhou and P. C. van Zijl, *Magn Reson Med*, 2009, **61**, 1441.