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)\textsuperscript{1}. The proteins were expressed in BL21 (DE3) E. coli after induction in Magic Media\textsuperscript{TM} 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°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\textsuperscript{2}, and a matrix size = 128×64, resulted in a resolution of 0.133×0.25 mm\textsuperscript{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\textsubscript{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\textsubscript{0} inhomogeneity, the absolute water resonance frequency shift was determined at each voxel using a modified WASSR method\textsuperscript{2}, with the same parameters as in CEST imaging except TR=1.5 sec and B\textsubscript{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\textsubscript{asym})=100×(S\textsuperscript{Δω} − S\textsuperscript{+Δω})/S\textsuperscript{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:
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\textsubscript{asym} values at different rf power, and b) increase in the obtained MTR\textsubscript{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\textsubscript{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\textsubscript{asym} values on the applied saturation pulse (B\textsubscript{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\textsubscript{1}=4000 ms. N=7 for each sampled protein.

Table S1: Number of arginine residues and the measured MTR\textsubscript{asym} value obtained at 1.8 ppm (1.25 mg/mL) at different B\textsubscript{1} powers.

<table>
<thead>
<tr>
<th></th>
<th>No. of arginines</th>
<th>MTR\textsubscript{asym} B\textsubscript{1}=2.4 mT</th>
<th>MTR\textsubscript{asym} B\textsubscript{1}=3.6 mT</th>
<th>MTR\textsubscript{asym} B\textsubscript{1}=4.7 mT</th>
<th>MTR\textsubscript{asym} B\textsubscript{1}=7.2 mT</th>
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</thead>
<tbody>
<tr>
<td>wt GFP</td>
<td>7</td>
<td>1.1±0.2%</td>
<td>1.9±0.2%</td>
<td>3.0±0.3%</td>
<td>4.3±0.2%</td>
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<tr>
<td>+36 GFP</td>
<td>20</td>
<td>1.9±0.2%</td>
<td>3.3±0.3%</td>
<td>4.6±0.3%</td>
<td>5.7±0.3%</td>
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<tr>
<td>+48 GFP</td>
<td>21</td>
<td>2.0±0.2%</td>
<td>3.4±0.2%</td>
<td>5.1±0.3%</td>
<td>6.1±0.1%</td>
</tr>
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References