A photoconvertible genetically encoded glutamate indicator for neuronal imaging

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Abstract

Synaptic transmission is a critical event of information processing in the brain. In particular, the specific patterns of neural activity at individual synapses can drive the growth, stabilization and elimination of synaptic connections. However, how complex patterns of neural activity at multiple synapses interact to drive changes in circuit connectivity remains poorly defined. Recently, improved genetically-encoded indicators of neuronal activity have allowed for functional measurements through optical recordings of calcium, glutamate and voltage. These applications have significantly advanced the field of systems neuroscience by permitting optical recordings in specific subpopulations of neurons; however, they don't provide information to link the activity to the structural changes. Here we developed a new class of photo-convertible genetically encoded glutamate indicator that would enable one to specifically record glutamate activity in subcellular compartments, such as single spines and axonal termini within the densely labeled neurons. We demonstrate the utility of this new glutamate indicator in cells and rat hippocampal neurons. We expect that our design strategy can be applied to other types of neurotransmitter indicators in the pipeline to allow multiplex imaging of synapses.

Introduction

Protein mEos photoconverts from green to red in the presence of 400nm light

iGluSnFR is a glutamate sensor in which cpGFP is inserted into a glutamate binding periplasmic binding protein from *E.coli*. Glutamate binding leads to intensity changes of fluorescence of cpGFP.

his-tag purified iGluSnFR, sat: 10mM glutamate Marvin et. al, Nature Methods 2013

Screening Methods

Promising glutamate sensing highlighter candidates with high dynamic range are selected via the formula:

$$\frac{\Delta F}{F} = \frac{\textit{Fluorescence with glutamate} - \textit{Fluorescence apo}}{\textit{Fluorescence apo}}$$

Promising integrator candidates that photoconvert more efficiently with glutamate than without glutamate are selected via the formula:

$$\frac{\Delta P}{P_o} = \frac{\frac{\Delta R_s}{G_{s\ initial}} - \frac{\Delta R_a}{G_{a\ initial}}}{\frac{\Delta R_a}{G_{a\ initial}}}$$

- P Photoconversion efficiency
- Δ Post-photconversion - pre-photoconversion
- R Red fluorescence
- G Green fluorescence
- s Glutamate saturated
- a Glutamate absent

Sensor screening via direct-evolution mutagenesis

Plot of photoconversion efficiency vs variant # obtained from screening candidates for glutamate integrators. Photoconversion efficiency improves after iterative rounds of directed evolution.

Selected References

Zhang, Mingshu, et al. "Rational design of true monomeric and bright photoactivatable fluorescent proteins." Nature methods 9.7 (2012): 727-729.

Marvin, Jonathan S., et al. "An optimized fluorescent probe for visualizing glutamate neurotransmission." Nature methods 10.2 (2013): 162-170.

Fosque, Benjamin F., et al. "Labeling of active neural circuits in vivo with designed calcium integrators." Science 347.6223 (2015): 755-760.

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Glutamate Highlighter

Concept

Purified Protein

Dotted lines are apo, solid lines are sat (10mM glut)

HEK-293T Cells

Selective photoconversion of transfected 46H1 displayed extracellarly on the membrane

In situ glutamate titration of extracellulary displayed sensors

Neurons

Linescans

Neuron (E18 dissociated culture) transfected with 49B3 and photoconverted

Sensitivity of Glutamate Highlighter in response to FP stimuli

$t_{1/2}$ on	green	red
20FP	120ms	120ms
10FP	50ms	50ms
1FP	15ms	15ms

$t_{1/2}$ off	green	red
20FP	800ms	800ms
10FP	550ms	550ms
1FP	380ms	380ms

Linescans acquired at 500Hz, bleach corrected and smoothed with a 3rd order Savitzky-Golay filter (width 15). Field potentials (FP) are administered at 30Hz.

Characterizing Glutamate Highlighter in dendritic spine in response to glutamate uncag-

Glutamate Integrator

Concept

Purified Protein

Dotted lines are apo, solid lines are sat (10mM glut)

Photoconversion rate of 75F4 vs glutamate concentration

HEK-293T Cells

To test photoconversion rate in situ, HEK-293T cells were transfected with extra-cellularly displayed 75F4 and photoconverted with the 405nm laser either without glutamate (apo) or in the presence of 100 μM glutamate (sat). High-resolution snaps were taken post-photoconversion in both green and red and then the R/G ratio was calculated. One example image from both apo and sat is shown above. A plot of 10 ROI's are show below.