NOVEL IGLUSNFR VARIANTS OPTIMISED FOR RAPID GLUTAMATE IMAGING

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Intensity-based glutamate-sensing fluorescent reporter iGlu-‘sniffer’ (iGluSnFR) is a useful tool for neuroscience that has enabled detection of glutamate release from single presynaptic terminals. However the probe’s fluorescence rise and decay kinetics appeared too slow to give an accurate readout of glutamate dynamics at the synapse during high frequency bursts. We thus generated novel variants with faster glutamate binding kinetics by mutation of amino acid residues coordinating glutamate at the binding site. Fast variants iGlu*f* and iGlu*u* have comparable brightness and fluorescence dynamic range to iGluSnFR. The *K*d for glutamate measured by equilibrium binding titration at 20 °C is increased from 33 M (iGluSnFR) to 137 M and 600 M (iGlu*f* and iGlu*u*, respectively). At 34 °C, *in vitro* dissociation rate measured by stopped-flow fluorimetry are increased up to 6-fold from 233 s-1 for iGluSnFR (*off*=4.3 ms) to 1481 s-1 for iGlu*u* (*off*=0.7 ms), making iGlu*u* the fastest glutamate fluorescent reporter to-date. At single presynaptic terminals stimulated at 100 Hz in hippocampal slice culture, iGlu*u* has 5-fold faster “*off*” rate (*off*=2.6 ms) than iGluSnFR, with the signal returning to baseline between each stimulus, revealing complete clearing of synaptic glutamate between high frequency release events. Glutamate neurotransmission shows pronounced depression during high frequency bursts that can be attributed to a depletion of presynaptic resources or desensitization of postsynaptic receptors. By comparing iGlu*u* signals and AMPA receptor currents, we show that synaptic depression during 100 Hz trains is entirely due to reduced glutamate release while the recovery after 500 ms has a postsynaptic component.

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