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Genetically Encoded Tool with Time-Resolved Fluorescence Readout for the Calcium Concentration Measurement

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Abstract : Here, we describe two variants of the calcium indicators based on the GCaMP sensitive core and BrUSLEE fluorescent protein (GCaMP-BrUSLEE and GCaMP-BrUSLEE-145). In contrast to the conventional GCaMP6-family indicators, these fluorophores are characterized by the well-marked responsiveness of their fluorescence decay kinetics to external calcium concentration both in vitro and in cellulo. Specifically, we show that the purified GCaMP-BrUSLEE and GCaMP-BrUSLEE-145 exhibit three-component fluorescence decay kinetics, with the amplitude-normalized lifetime component (t3*A3) of GCaMP-BrUSLEE-145 changing four-fold (500-2000 a.u.) in response to a Ca^{2+} concentration shift in the range of 0—350 nM. Time-resolved fluorescence microscopy of live cells displays the two-fold change of the GCaMP-BrUSLEE-145 mean lifetime upon histamine-stimulated calcium release. The aforementioned Ca^{2+}-dependence calls considering the GCaMP-BrUSLEE-145 as a prospective Ca^{2+}-indicator with the signal read-out in the time domain.

Keywords : calcium imaging, fluorescence lifetime imaging microscopy, fluorescent proteins, genetically encoded indicators
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