Improvement of the kinetics of red fluorescent calcium indicators

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Genetically encoded calcium indicators (GECIs) are widely used for monitoring calcium signalling in various cell types. They are based on calmodulin (CaM), circular-permuted fluorescent protein (cpEGFP or others) and the light-chain myosin kinase peptide RS20. Although genetically engineering has led to a broad variety of different GECIs and also red probes suitable for optogenetics, there are only few variants with fast kinetics [1]. We have performed mutations on two red fluorescent GECIs, jRCaMP1a and jRGECO1a [2], to weaken the interaction between CaM and the RS20 target peptide, to fasten the kinetics of these slow probes.

In total we made and biophysically characterized 17 variants. At physiological ionic strength and 20 °C, jRCaMP1a shows a biexponential fluorescence rise with rates 52±1 (47 %) and 2.3±0.1 s^{-1} (53 %) and a dynamic range of 6.8±0.3. Fluorescence decay is biexponential with a fast (2.2±0.1 s^{-1} (17 %)) and slow phase (2.2±0.1 s^{-1} (17 %) and 0.32±0.01 s^{-1} (83 %)). The variant termed jRCaMP1a$_{fast}$ has similar rise kinetics to jRCaMP1a with rates 15±1 (47 %) and 2.1±0.1 s^{-1} (53 %) but decays 21-fold faster (6.9 s^{-1}) with dynamic range of 4.5±0.2.

jRGECO1a (dynamic range 12.3±1.2) has single exponential kinetics with rise and decay rates 150±3 s^{-1} and 4.3±0.1 s^{-1}, respectively. The variant termed jRGECO1a$_{fast}$ has a similar rise rate (150±3 s^{-1}) with a 5-fold faster bi-phasic decay (k_{off}: 25±2 (85 %) and 1.5±0.1 (15 %)) and dynamic range (12.9±0.8). The variant termed jRGECO1a$_{ultrafast}$ has slower rise kinetics (53±2 s^{-1}) but a 100-fold faster decay (k_{off}: 515±80 (80 %) and 7±1 (20 %)) and a dynamic range of 6.9±0.1.

The faster kinetics and preserved dynamic ranges of the novel red GECIs make them useful imaging tools.

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References

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