**Fast-decay variants of red-fluorescent genetically-encoded calcium indicators**

Silke Kerruth1, Catherine Coates1 and Katalin Török1

1St. George's University of London, Cranmer Terrace, SW17 0RE, London, United Kingdom.

The time-course of intracellular Ca2+ transients is hard to assess due to buffering and signal integrating interactions. Genetically-encoded calcium indicators (GECI) have proven useful for monitoring Ca2+ transients in living cells and organisms. However, Ca2+ indicators with high Ca2+ affinity and slow decay kinetics themselves integrate Ca2+signals, and furthermore may become saturated before peak [Ca2+] is reached. Thus, for more faithful tracking of rapid Ca2+ dynamics, probes with faster off-kinetics are required1,2. Red-fluorescent GECI have been developed with the view of multicolour imaging and optogenetic applications3. Here we report novel fast-decay variants of red-fluorescent genetically-encoded Ca2+ indicators jRGECO1a and jRCaMP1a3 with up to 8-fold (*t*1/2 of 6.4 ms) and 13-fold (*t*1/2of 33 ms) faster *in vitro* decay kinetics (37 °C), respectively. Fast-decay jRGECO1a and jRCaMP1a variants retain comparable fluorescence brightness and dynamic range values to their parent proteins. The fluorescence dynamic range of the brighter mApple-based jRGECO1a variants is stable between pH 6.5 and 7.5, but declines above pH 7.5 to a Ca2+-independent fluorescent state. In contrast, the less bright jRCaMP1a variants, based on mRuby, are stable over the pH range of 6.5 to 10. Red-fluorescent GECI, like their green-fluorescent counterparts, are characterised by high cooperativity to Ca2+, and complex kinetic patterns of Ca2+-dependent fluorescence response with a limiting *on*-rate. However, the fast-decay variants of jRGECO1a and jRCaMP1a reveal 8-fold faster ATP-evoked Ca2+ transients compared to their parent proteins in HEK293T cells, showing the benefits of fast-decay red-fluorescent GECI indicators for monitoring Ca2+ dynamics in living cells.

This work is funded by BBSRC grant BB/M02556X/1 to K.T.

References

[1] Helassa et al., **2015** *Scientific Reports*, 5:15978.

[2] Helassa et al., **2016** *Scientific Reports*, 6:38276.

[3] Dana et al., **2016** *eLife*, 5:e12727.