Fast-decay variants of red-fluorescent genetically-encoded Ca^{2+} sensors

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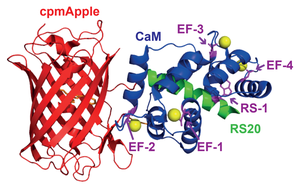
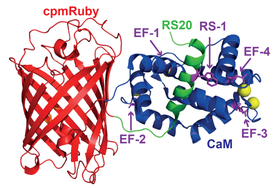
Why make fast red probes?

- **Why red?** Red-fluorescent genetically-encoded Ca^{2+} indicators (RGECIs) monitor Ca^{2+} transients while cells can be simultanously excited with blue-light for optogenetics.
- **Challenge:** Current RGECIs[1] have slow response, faster variants needed to monitor action potential firing
- **Strategy:** To weaken the CaM and RS20 peptide interaction by mutating the RS20 peptide and disabling the CaM EF hands of jRGECO1a and jRCaMP1a[2,3].

RGECO1 (PDB: 4I2Y)



RCaMP1h (PDB: 3U0K)

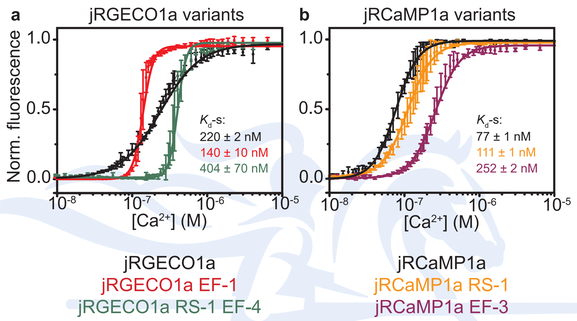


Biophysical characterization and kinetics

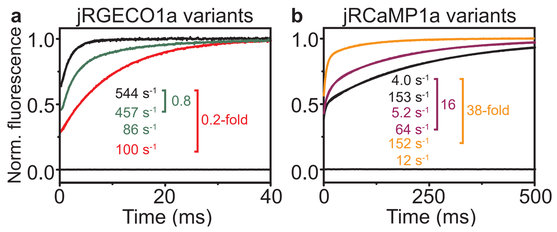
Fluorescence enhancement

Variant	jRGECO1a	jRGECO1a EF-1	jRGECO1a RS-1 EF-4	jRCaMP1a	jRCaMP1a RS-1	jRCaMP1a EF-3
$F_{+Ca^{2+}} / F_{-Ca^{2+}}$	14 ± 4	10 ± 5	14 ± 6	7 ± 3	6 ± 2	6 ± 2

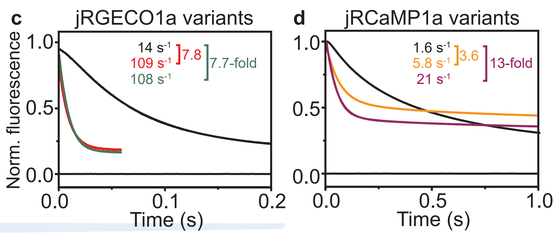
Equilibrium Ca^{2+} titrations (20 °C)



Association kinetics (37 °C)



Dissociation kinetics (37 °C)



Experimental Details

Ca^{2+} equilibrium titrations:

- RGECIs in assay buffer (50 mM K^+-HEPES, 100 mM KCl, 2 mM $MgCl_2$, pH 7.5) + 10 mM EGTA
- continous titration with 325 mM $CaCl_2$

Ca^{2+} transients in HEK293T cells:

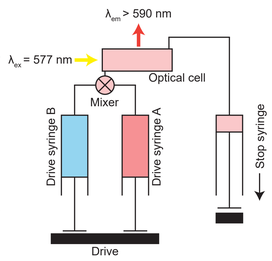
- RGECIs expressed in HEK293T cells
- after 16-48 h imaged on confocal Zeiss AxioObserver Z1 at 37 °C (CMOS 2 s interval, λ_{ex}=568 nm)
- Ca^{2+} transients triggered with 50 µM ATP

Association kinetics:
RGECIs in assay buffer + 10 mM EGTA were rapidly mixed with increasing $CaCl_2$ in assay buffer

Dissociation kinetics:
RGECIs in assay buffer + 1 mM $CaCl_2$ were rapidly mixed with12.5 mM EGTA (conc. in mixing chamber)

Free $[Ca^{2+}]$ calculated by Maxchelator.

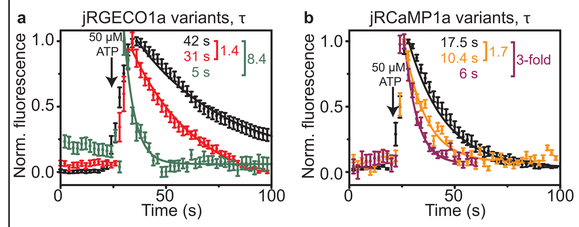
Stopped-flow fluorimetry



Summary

- Faster RGECI variants are developed without significant loss of brightness and fluorescence dynamic range
- jRCaMP1a RS-1 compared to jRCaMP1a: Limiting association rate 38-fold faster
- jRGECO1a RS-1 EF-4 and jRCaMP1a RS-1 compared to jRGECO1a and jRCaMP1a:
  - Dissociation kinetics 8-fold and 13-fold faster, respectively
  - ATP-induced cell Ca^{2+} transient decay time courses are 8-fold and 3-fold faster, respectively
- Rate constants are fitted to two-step model for probes which show rapid binding followed by limiting isomerisation

Cellular Ca^{2+} dynamics



Ca^{2+} dependence of association kinetics

Observed rates increase with increasing $[Ca^{2+}]$ and showing sigmoidal dependence.

The k_{obs} were fitted to a model, with an equilibrium Ca^{2+} binding followed by an isomerization to form a fluorescent state.

(**d**) Irreversible isomerisation:

$$k_{obs} = \frac{k_{+2}K_1[Ca^{2+}]^n}{1 + K_1[Ca^{2+}]^n}$$

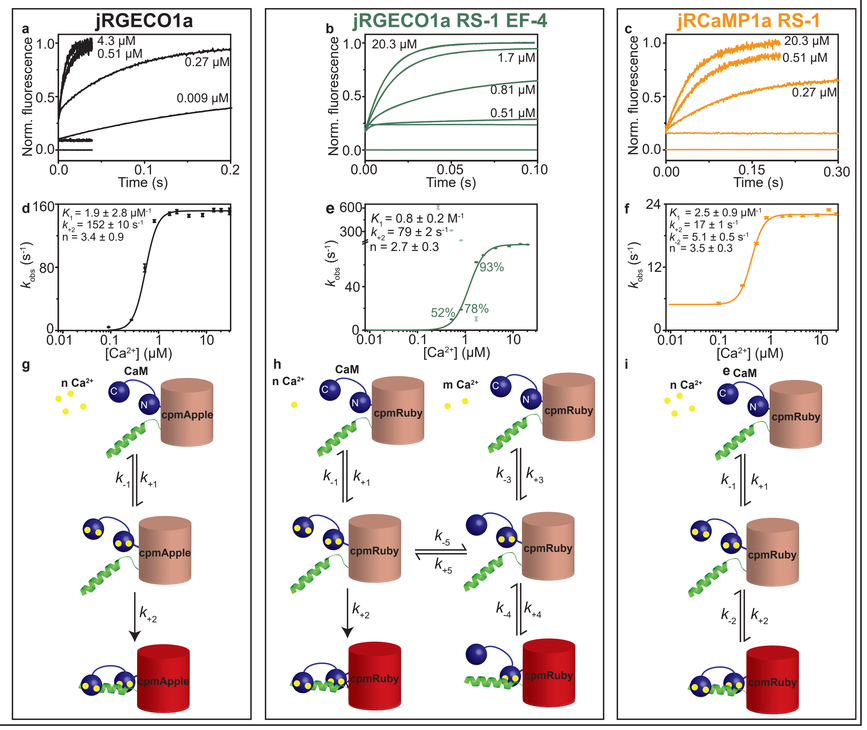
(**e**) Complex reaction with two fluorescent paths

$$k_{obs} = \frac{k_{+2}K_1[Ca^{2+}]^n}{1 + K_1[Ca^{2+}]^n}$$

$$k_{obs} = \frac{k_{+4}K_3[Ca^{2+}]^m}{1 + K_3[Ca^{2+}]^m} + k_{-4}$$

(**f**) Reversible isomerisation:

$$k_{obs} = \frac{k_{+2}K_1[Ca^{2+}]^n}{1 + K_1[Ca^{2+}]^n} + k_{-2}$$



References

[1] Dana et al., eLife, 2016.
[2] Helassa et al., Sci. Rep., 2015
[3] Helassa et al., Sci. Rep., 2016

Funding

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