

Development of fast-GCaMP indicators for neuronal spike counting

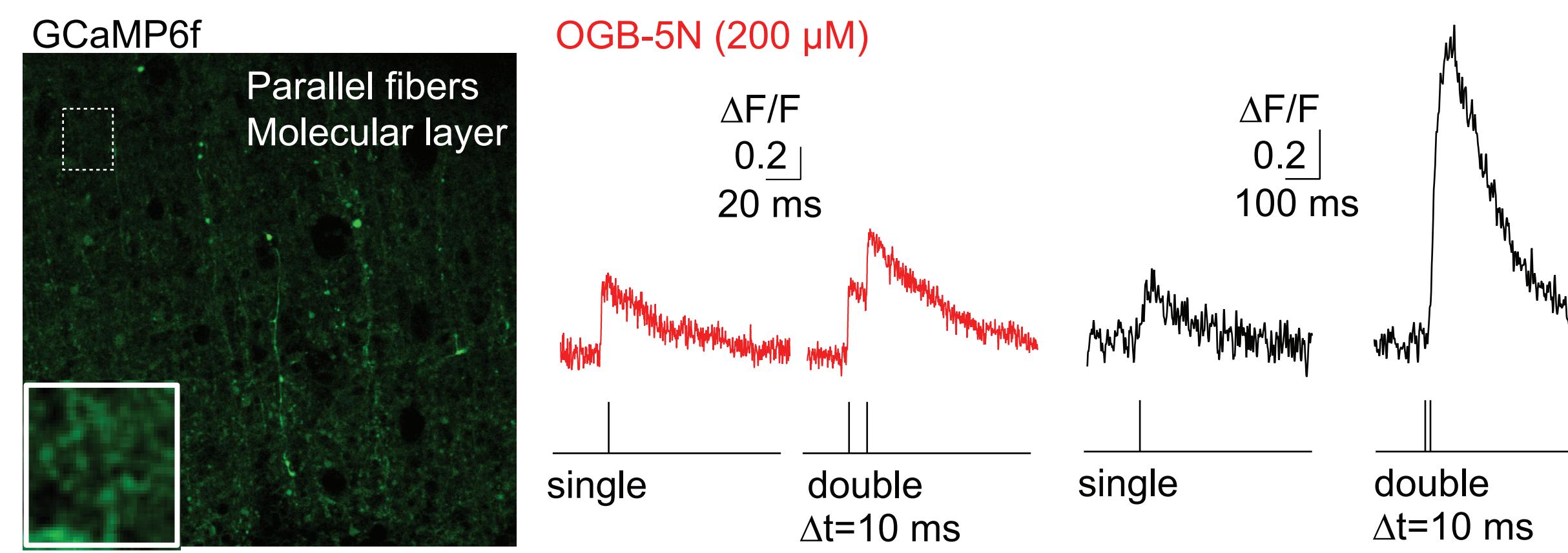
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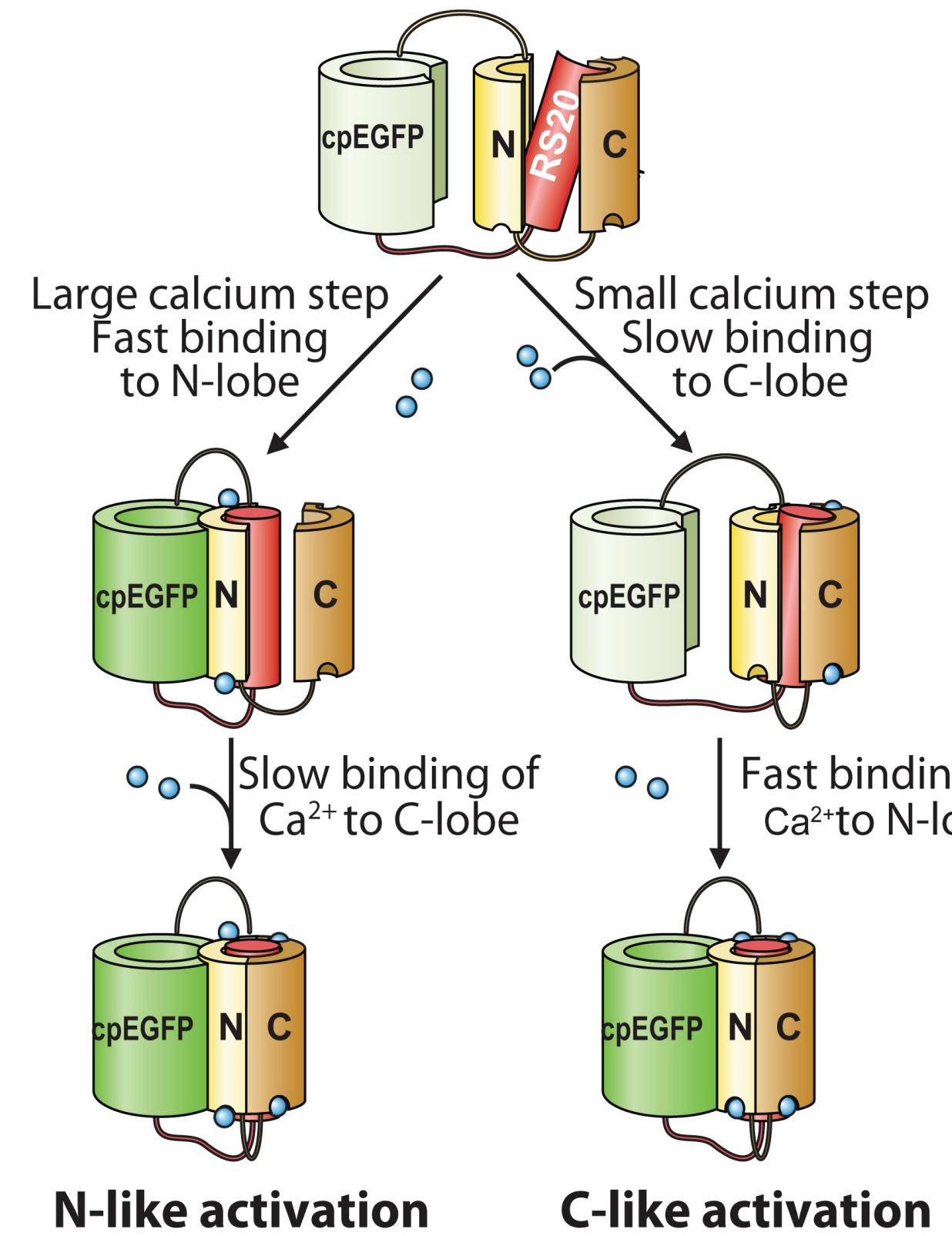
Calcium Transient Kinetics

Fluorescent calcium sensor proteins such as GCaMP (GFP-Calmodulin fusion protein) have emerged as a powerful technology for monitoring neural circuitry. However GCaMPs report slow signals when expressed in cell bodies.



In axonal boutons, calcium dynamics involve fast rise kinetics and decay kinetics with $t_{1/2}$ of ~30 ms. Compared to synthetic Ca indicators, GCaMP6f is not kinetically sensitive enough to separate high frequency pulses and temporally filters decay signals. We sought to improve upon these sluggish kinetics through structure-guided mutation of GCaMP6f.

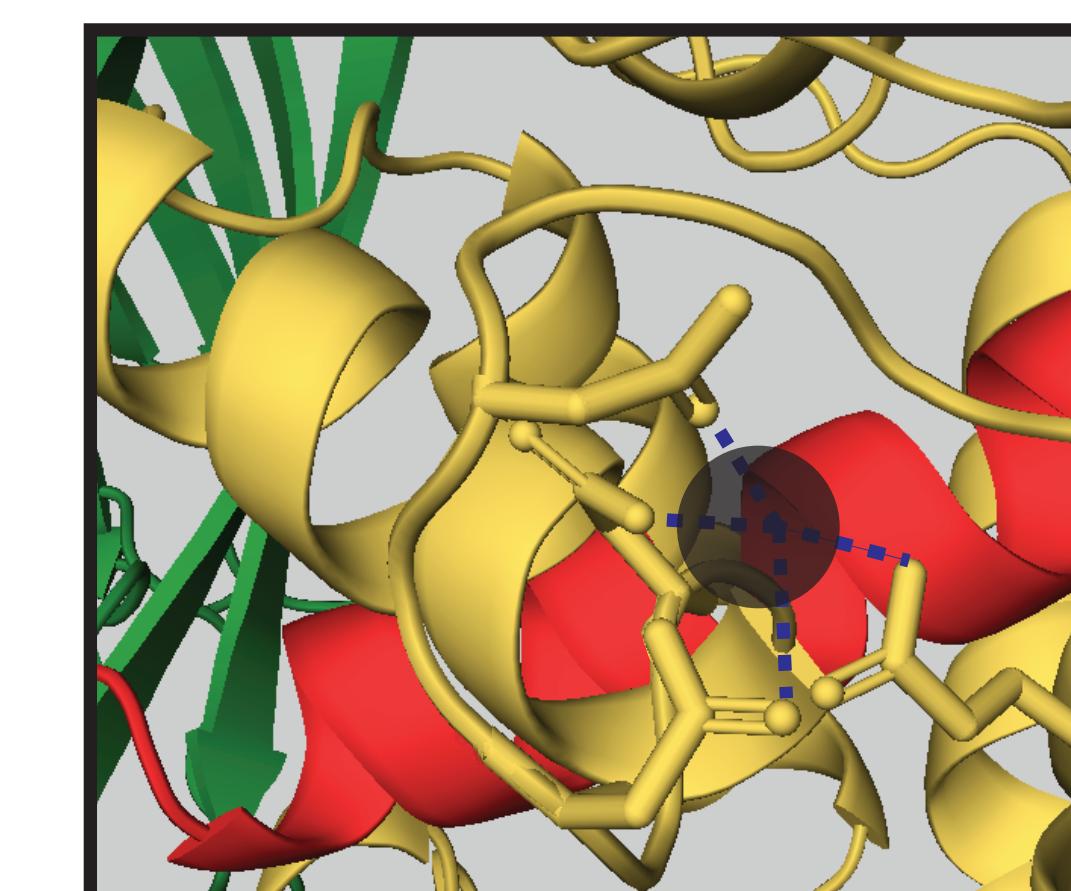
Mutagenesis Strategy



Accelerating Rise Kinetics

We mutated C-lobe, EF hand binding sites to:

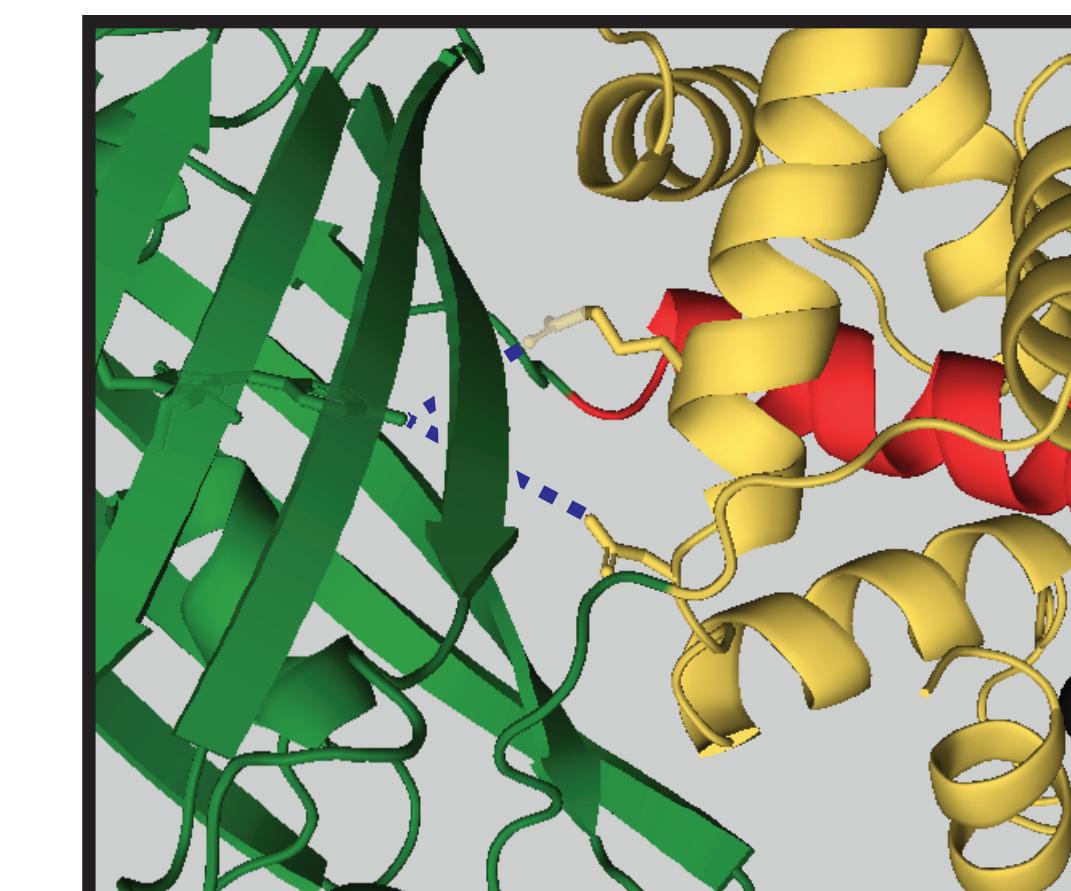
- ◆ stiffen the binding loops (Gly → Pro)
- ◆ convert chelating residues from basic to acidic
- ◆ inactivate chelating residues through non-polar substitutions



Accelerating Decay Kinetics

We sped calcium release by altering the polarity of RS residues with solvent interactions to:

- ◆ stabilize Ca-free conformation
- ◆ destabilize Ca-bound conformation



Biophysical and Kinetic Responses

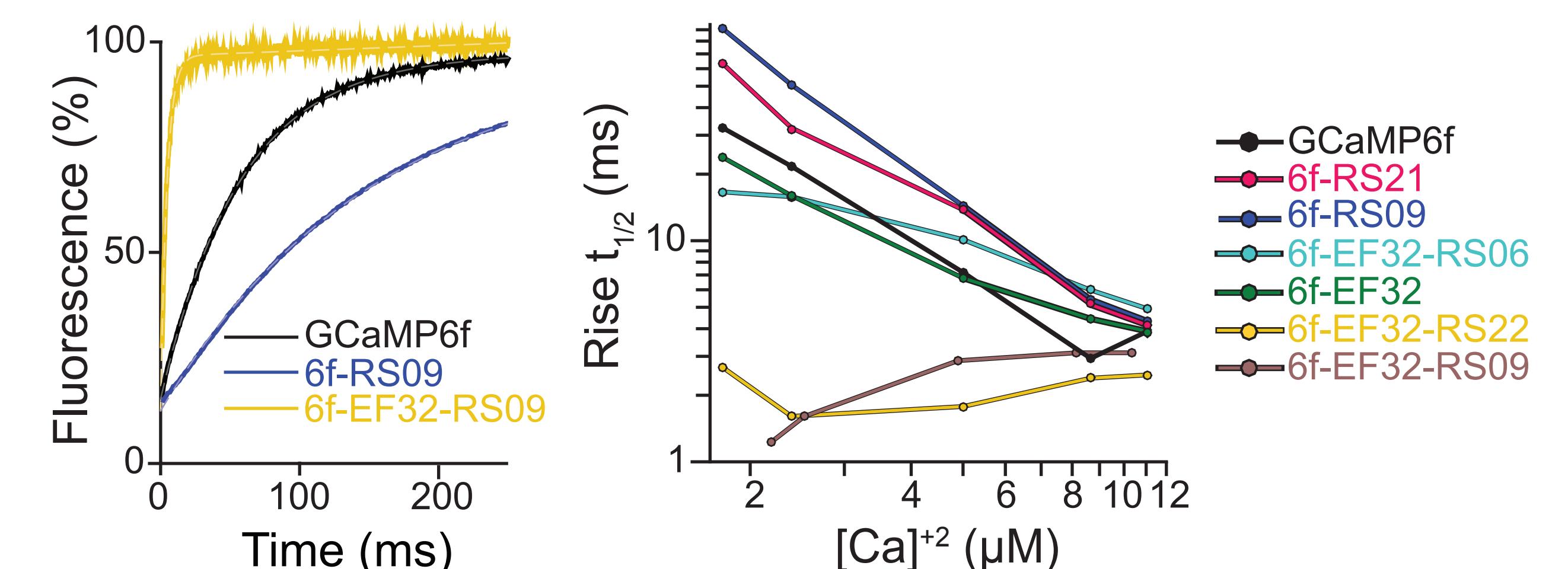
Fluorescence and Affinity

Variant*	K_d (nM)	n_H	Relative f_{max}	R_f
GCaMP6f	310 ± 20	2.19 ± 0.10	1	27 ± 4
6f-RS21	350 ± 40	2.85 ± 0.20	0.60 ± 0.08	20 ± 3
6f-RS09	540 ± 40	2.42 ± 0.08	0.72 ± 0.10	29 ± 4
6f-EF32-RS06†	640 ± 30	2.53 ± 0.26	0.84 ± 0.12	29 ± 4
6f-EF32	740 ± 30	2.49 ± 0.08	0.82 ± 0.12	24 ± 3
6f-EF32-RS22	2500 ± 560	2.41 ± 0.38	0.85 ± 0.12	20 ± 3
6f-EF32-RS09	2600 ± 400	2.25 ± 0.26	0.90 ± 0.13	21 ± 3

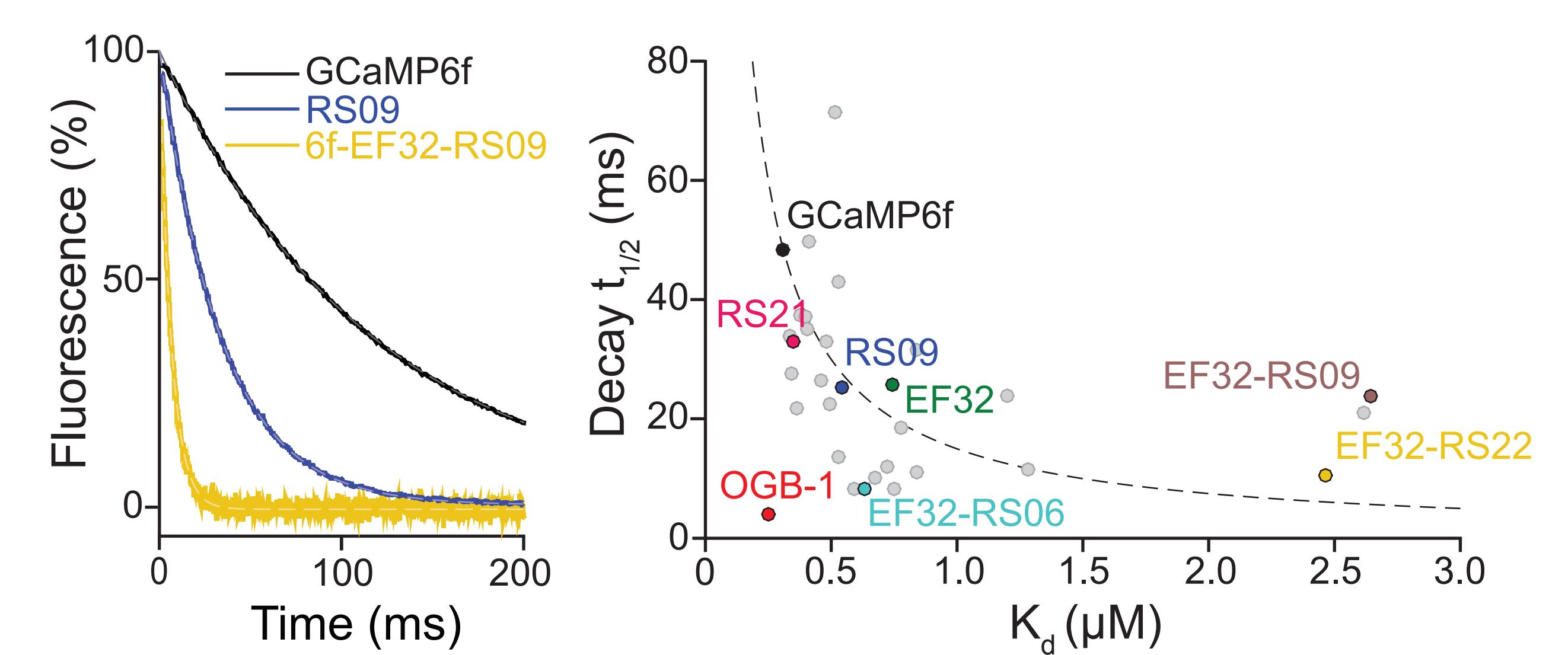
*Nomenclature from Sun et al. (2013) Nat. Comm.

†D395A; Helassa et al. (2015) Sci. Rep.

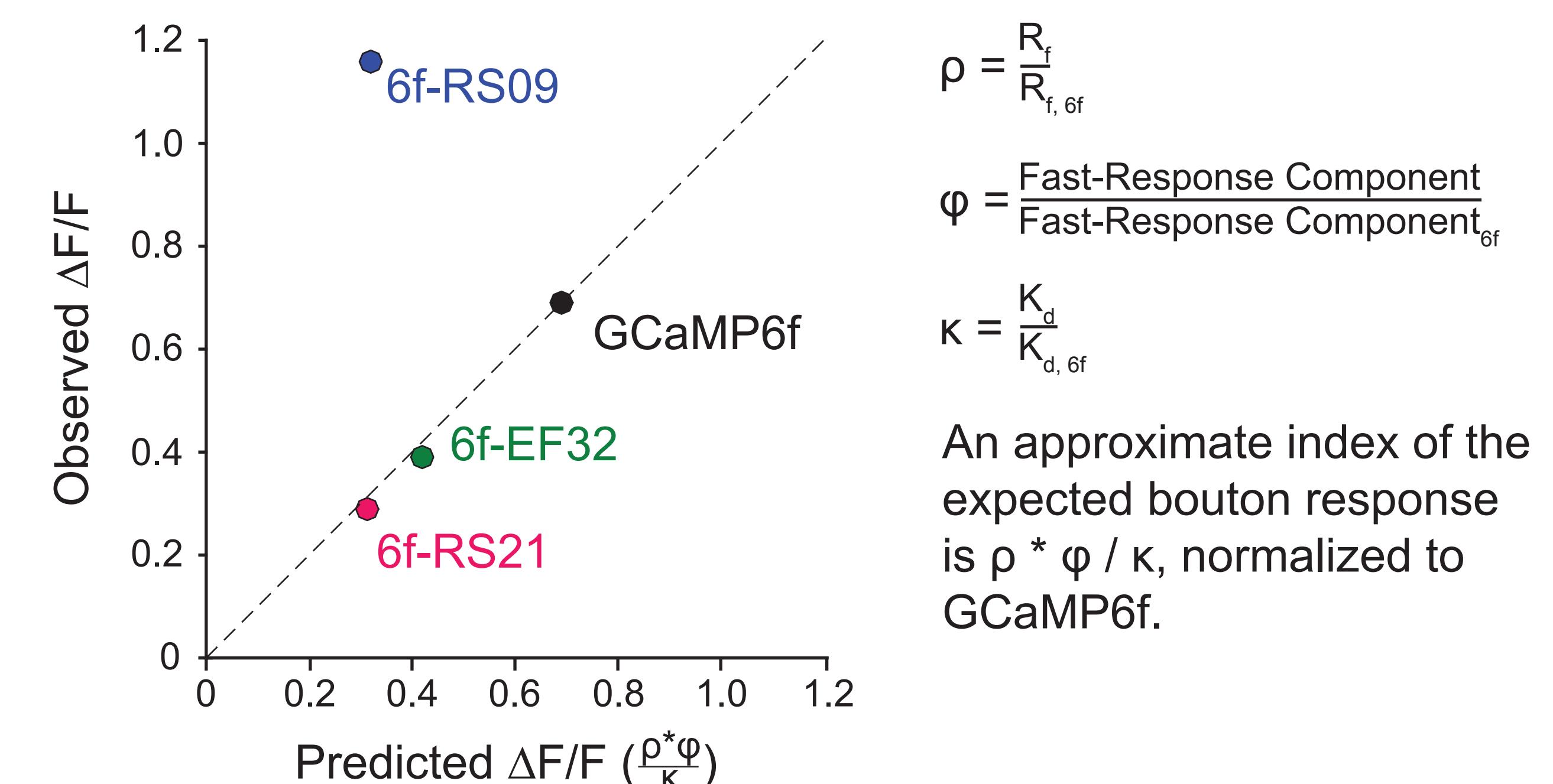
Rise Kinetics



Decay Kinetics



Comparison with Bouton Measurements



$$\rho = \frac{R_f}{R_{f,6f}}$$

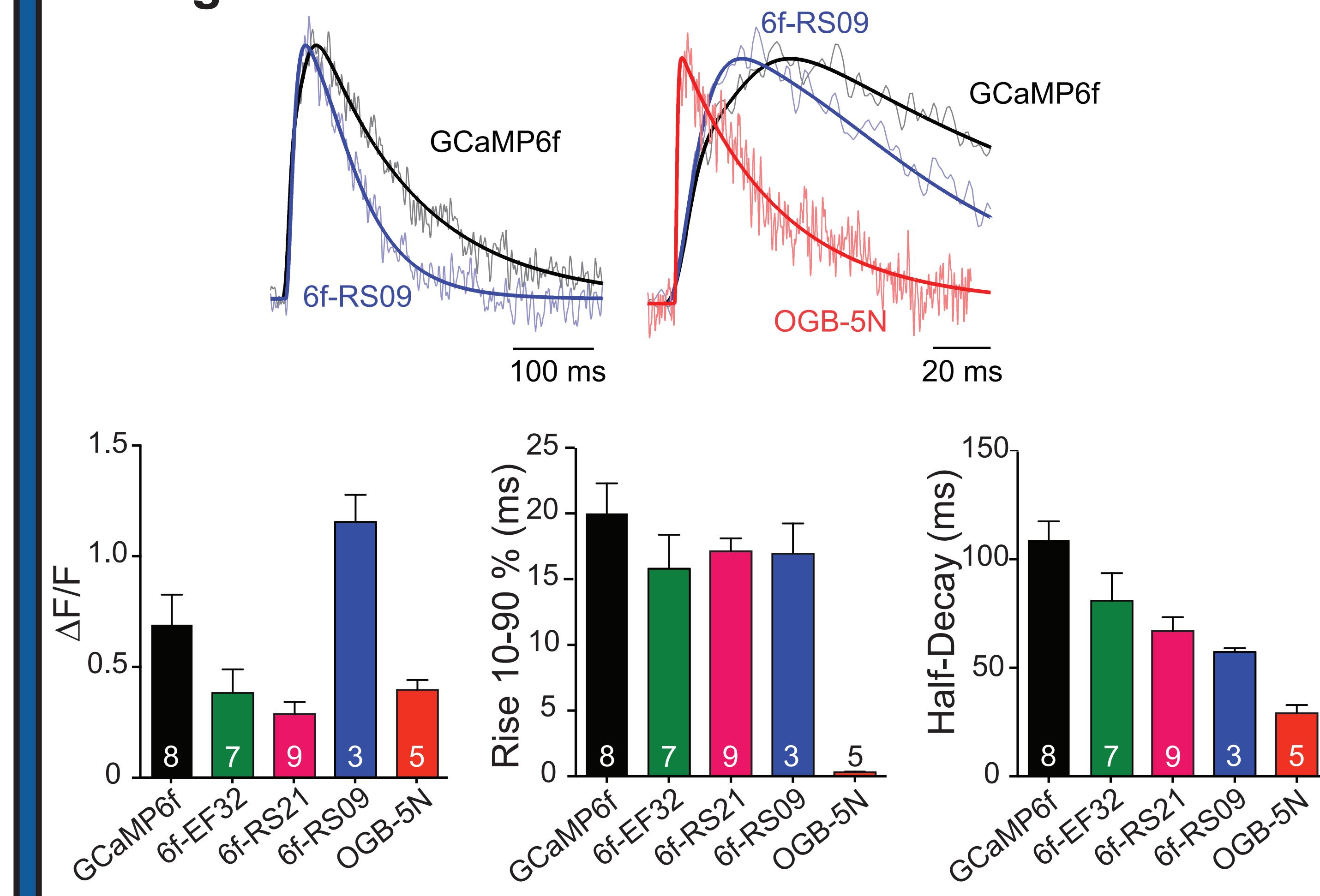
$$\varphi = \frac{\text{Fast-Response Component}}{\text{Fast-Response Component}_{6f}}$$

$$\kappa = \frac{K_d}{K_{d,6f}}$$

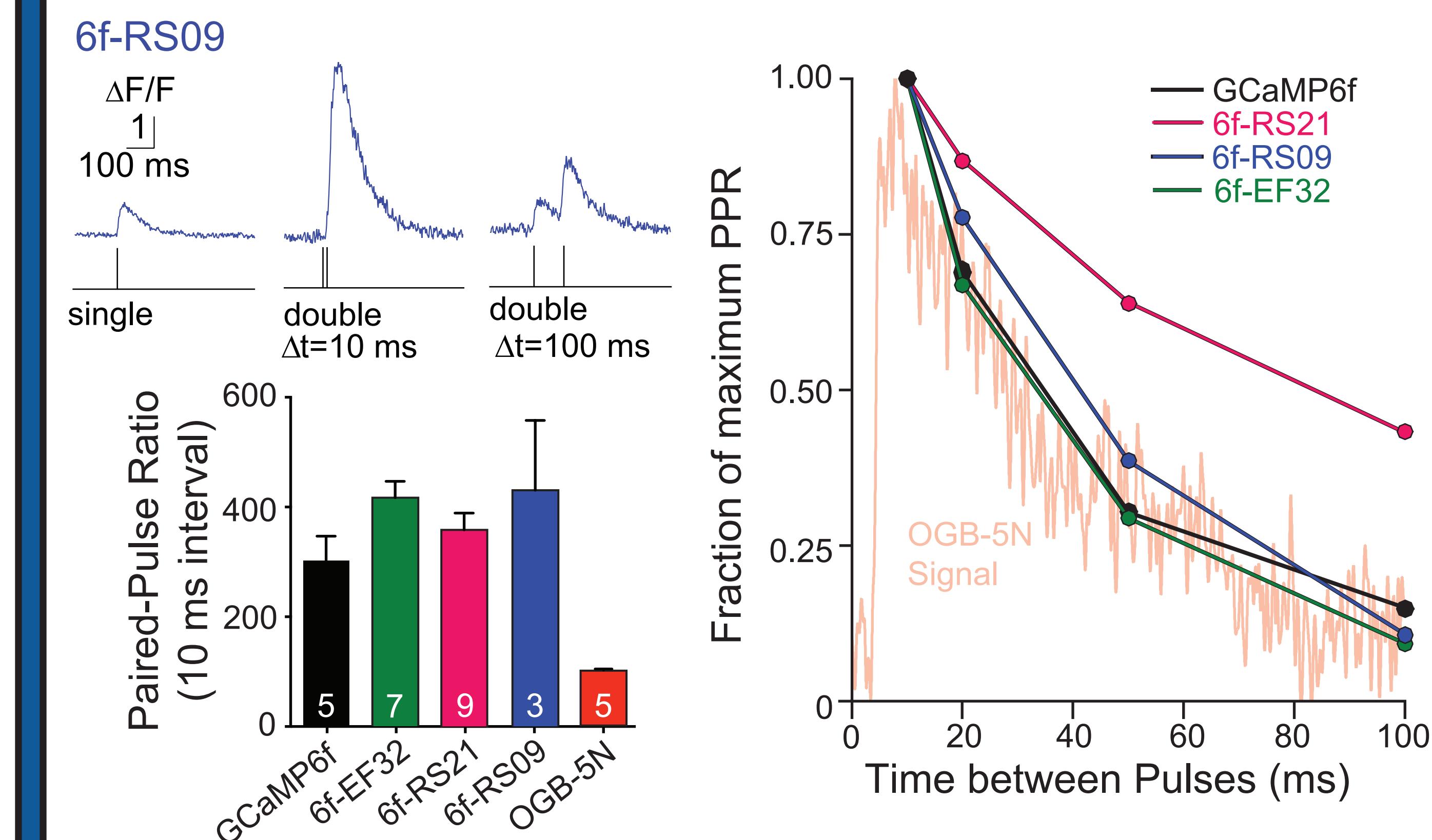
An approximate index of the expected bouton response is $\rho * \varphi / \kappa$, normalized to GCaMP6f.

Two-Photon Imaging of Axonal Boutons

Single Pulse



At short inter-spike intervals, signals become non-linear



Conclusions

- ◆ Calcium binding ("EF") and intramolecular domain ("RS") variants of GCaMP6f can accelerate probe response dynamics to the time scale of bouton signals.
- ◆ Steady-state and kinetic properties of GCaMP variants can be used to estimate possible bouton signal properties.
- ◆ Using GCaMP6f-RS09, bouton signals have rise and fall times that may permit monitoring at ~20 Hz.
- ◆ With newer variants, single-spike resolution may approach physiology-limited rates of ~50 Hz.