OPTICAL MONITORING OF NEURONAL FIRING RATES WITH NEW, FAST-RESPONDING GCaMP VARIANTS

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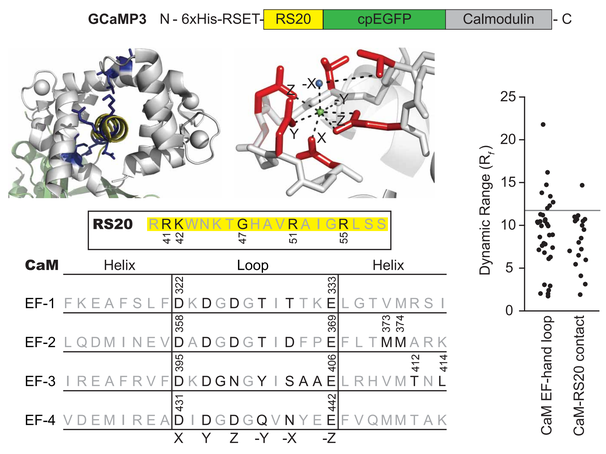
ABSTRACT

Although fluorescent genetically encoded calcium indicator (GECI) proteins provide a powerful tool for monitoring neuronal activity, they still have significant performance limitations compared with synthetic indicators such as Oregon Green BAPTA-1 (OGB-1). Because of high cooperativity originating from an EF hand protein-based detection mechanism, a given GECI is only sensitive to a small part of a neuron's likely calcium concentration range, which can exceed 1 μM during intense activity. GECIs also have up to 100-fold slower reponse kinetics than OGB-1, whose τ_{on} is ~1 ms to a step increase in calcium and τ_{off}=~7 ms to a step decrease. Overcoming limitations in range and kinetics is a key step toward monitoring spike times and firing rates in cell-type-specific brain circuits.

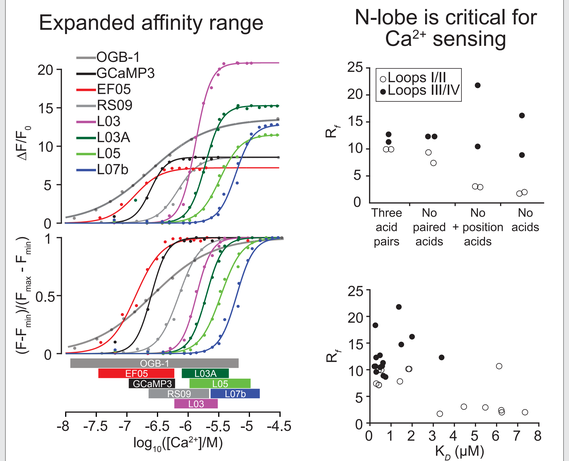
We are engaged in structure-based design to vary the affinity and accelerate the response kinetics of a popular GECI, GCaMP3 (Nakai et al. 2001 Nat. Biotechnol. 19:137; Tian et al. 2009 Nat. Methods 6:875). We have designed more than 50 variants by targeted mutation of GCaMP3's calmodulin domain and its intraprobe binding partner, RS20. In our cuvet characterizations of purified protein, we have (1) attained a nearly 40-fold (0.16-6 μM) range of K_D without impairing per-molecule brightness, and (2) made stopped-flow biochemical measurements in which off-responses (i.e. to decreasing steps in calcium) are more than 10 times faster than any other published GECI. Most of the gap in off-response speed between G-CaMP3 and OGB-1 could be closed without perturbing K_D (see Figure panel A).

We are using two-photon laser scanning microscopy to characterize our most promising variants in cultured sympathetic neurons by pseudorabies virus (PRV) infection, Drosophila neuromuscular junctions by transgenesis, and cerebellar neurons in brain slices by adeno-associated virus (AAV) infection (Figure panels B/C/D). Our long-term goal is to generate and identify variants and combinations of variants that detect single action potentials and/or firing rate modulations in the 1-100 Hz range.

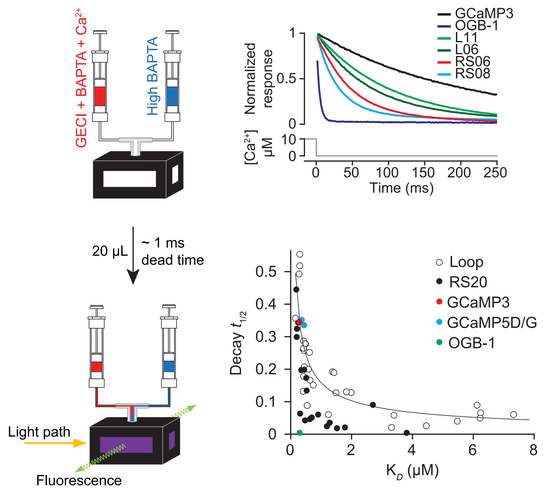
1 STRUCTURE-ORIENTED OPTIMIZATION



2 CONTROLLING AFFINITY



3 FASTER OFF-RESPONSES TO DECREASES IN CALCIUM

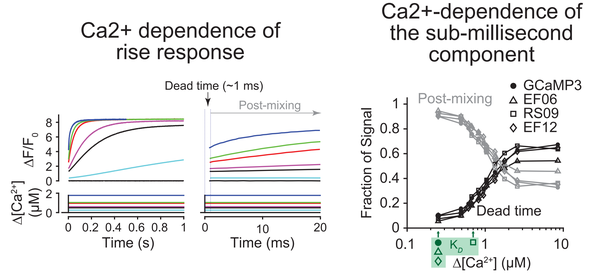


4 A RANGE OF AFFINITY AND OFF-RESPONSE TIMES

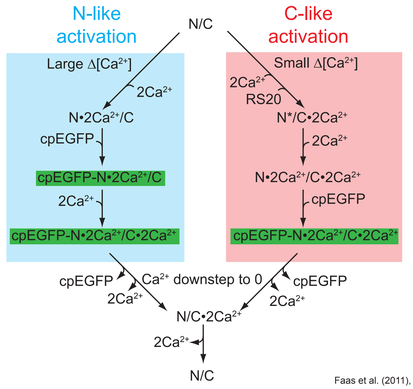
Variant	Altered domain	K_D (μM)[1]	R_f	Decay $t_{1/2}$ (ms)[2]
OGB-1	-	0.24	14.0	5
GCaMP3	-	0.25	12.0	150
EF05	Loop	0.16	6.8	183
L03	Loop	1.37	21.8	80
L03A	Loop	1.99	16.2	52
L05	Loop	3.39	12.3	22
L07b	Loop	6.12	13.8	35
RS06	CaM-Helix	0.31	6.5	34
RS05	CaM-Helix	0.47	8.5	27
RS08	CaM-Helix	0.63	10.0	23
RS09	CaM-Helix	0.69	9.5	25
RSd03	RS20	0.90	11.1	22
RSd05	RS20	1.19	10.7	10
RSd06	RS20	1.78	10.6	7

[1]23°C; [2]37°C

5 A LOW-AFFINITY MODE FOR RAPID ON-RESPONSES

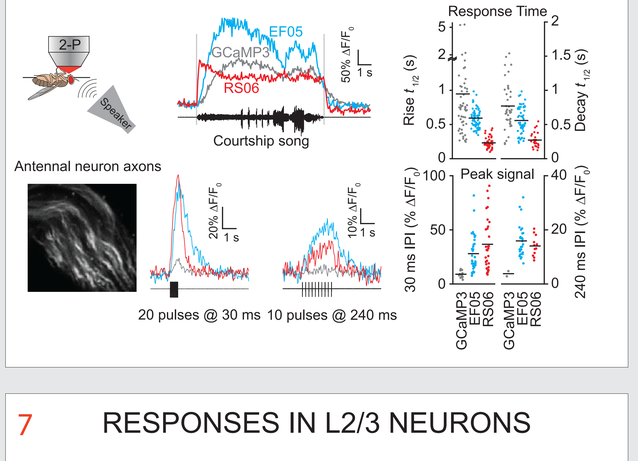


A TWO-PATH MODEL FOR GCaMP ACTIVATION

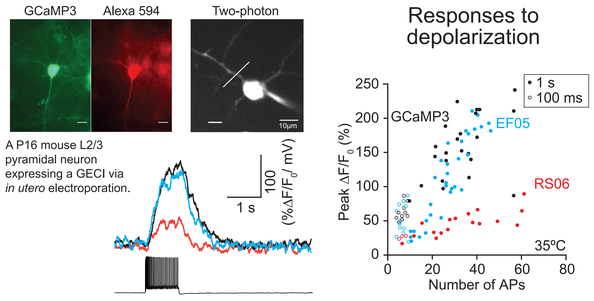


Faas et al. (2011), Nat Neurosci (14)301-4.

6 AXON RESPONSES IN *DROSOPHILA*



7 RESPONSES IN L2/3 NEURONS



SUMMARY

1. Of over 50 variants made, 32 have dynamic ranges within 0.7-1.8 times that of GCaMP3.
2. Variants achieved nearly 40-fold (0.16-6 μM) ranges of K_D.
3. We achieved a 4- to 50-fold improvement off-response times.
4. Fast, low affinity component to the rise response in all variants tested.
5. In *Drosophila*, variants performed as expected from K_D, with fast variants showing a ~3-fold faster rise and decay of signals.

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