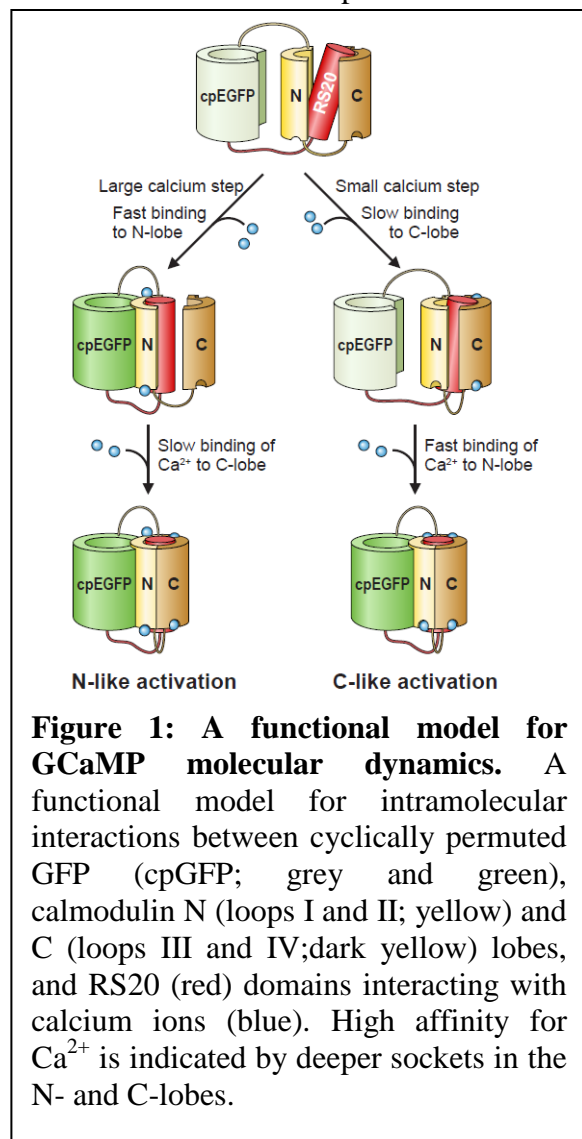


**Progress Report**  
**McKnight Foundation Technological Innovations in Neuroscience Award**  
**Transcending the Dynamic Limits of Genetically Encodable Calcium Indicators**  
**Samuel S.-H. Wang, Princeton University**  
**August 2013**

*With support from the McKnight Foundation, we have developed Fast-GCaMPs, the fastest-responding calcium indicators made to date. We have published our first paper announcing these probes (Sun, Badura et al. Nature Communications 4:2170). Now we are entering the next phase of development, combining Fast-GCaMP mutations with the recently developed GCaMP6f, which is brighter but not as fast-responding. We plan to apply the resulting Fast-GCaMP6f variants to the problem of monitoring cerebellar networks in awake mice. We will quantify activity in cerebellar granule cells during behavior to probe David Marr's famous proposal that these cells express a sparse activity code.*

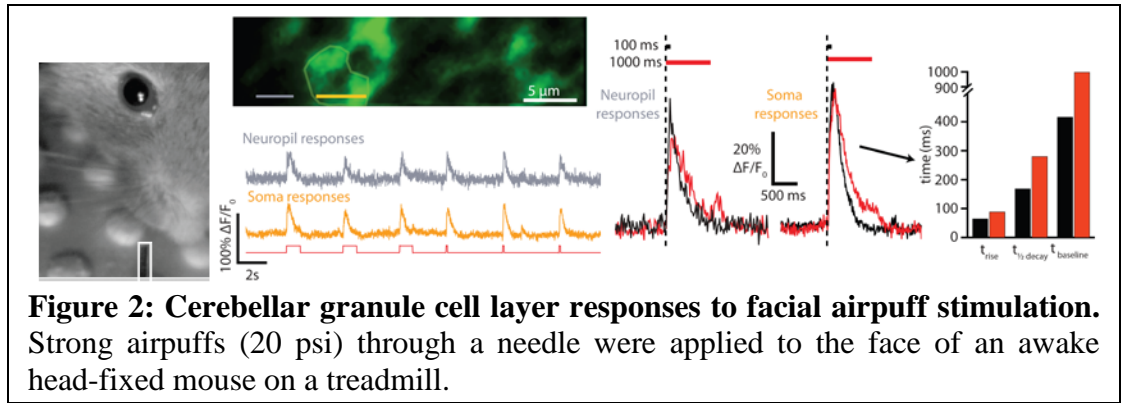
**Fast GCaMPs for improved tracking of neuronal activity.** Starting from an initial sensory event, brains can act within a split second. Monitoring the intervening chain of events requires the ability to detect neuronal signals with millisecond resolution in individual neurons and synapses. Useful for this purpose are genetically encodable calcium indicator (GECI) proteins, which take advantage of the fact that calcium ( $\text{Ca}^{2+}$ ) enters neurons whenever they are active. However, GECIs in use today are hampered by slow response times and sensitivity to only a narrow range of  $\text{Ca}^{2+}$  concentrations.



To address this situation, we have improved the GFP-based calcium indicator protein GCaMP3, a popular GECI. We have identified three performance-limiting features of GCaMP3. First, we find that affinity is regulated by the calmodulin domain's  $\text{Ca}^{2+}$ -chelating residues. Second, we find that off-responses to  $\text{Ca}^{2+}$  are rate-limited by dissociation of the RS20 domain from calmodulin's hydrophobic pocket. Third, we find that on-responses are limited by fast binding to the N-lobe at high  $\text{Ca}^{2+}$  and by slow binding to the C-lobe at lower  $\text{Ca}^{2+}$ . These principles are diagrammed in **Figure 1**.

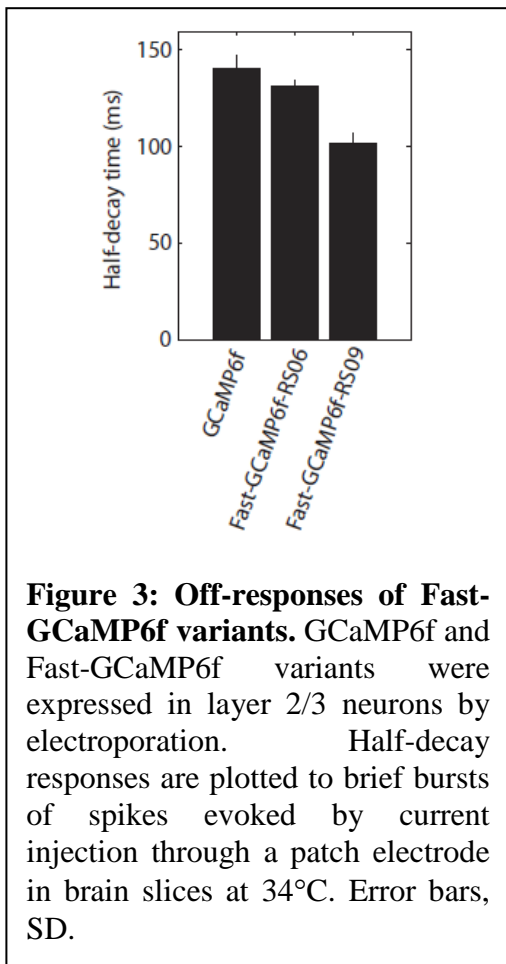
Our Fast-GCaMPs have up to 20-fold accelerated off-responses and have a 200-fold range of  $K_D$  (see Sun et al., Figure 3). The latter property opens the possibility of coexpressing multiple variants to span an expanded concentration range. This is important because biological calcium signals inside cells can range from  $0.1 \mu\text{M}$  to  $>10 \mu\text{M}$ . As a test of function, we have shown that Fast-GCaMPs track natural song in *Drosophila* auditory neurons and generate rapid responses in mammalian neurons, supporting the utility of our approach.

**In vivo calcium imaging.** We are using GCaMP6f, a recently developed calcium indicator protein that is brighter than GCaMP3. Using two-photon microscopy we observe activity



**Figure 2: Cerebellar granule cell layer responses to facial airpuff stimulation.** Strong airpuffs (20 psi) through a needle were applied to the face of an awake head-fixed mouse on a treadmill.

of cerebellar granule cell layer neurons (GC layer neurons) in awake head-fixed mice. We can monitor activity to observe signals associated with sensory processing and learning. In GC layer neurons, brief (100 ms) airpuffs delivered to the whisker area of the face evoke large signals that peak at the end of the airpuff and decline with a half-decay time of 170-300 ms (**Figure 2**).



**Figure 3: Off-responses of Fast-GCaMP6f variants.** GCaMP6f and Fast-GCaMP6f variants were expressed in layer 2/3 neurons by electroporation. Half-decay responses are plotted to brief bursts of spikes evoked by current injection through a patch electrode in brain slices at 34°C. Error bars, SD.

**Future directions: Brighter variants and accelerating the on-response.** Recently, we have put some of our most promising Fast-GCaMP mutations, termed RS06 and RS09, into GCaMP6f, with promising results. Fast-GCaMP6f-RS06 has 70% the maximal brightness of GCaMP6f, and Fast-GCaMP6f-RS09 has >90% the brightness of GCaMP6f. Furthermore, in layer 2/3 neocortical neurons in brain slices, Fast-GCaMP6f-RS09 shows off-responses that are 30% faster than GCaMP6f (**Figure 3**).

Next we plan to express Fast-GCaMP6f variants in cerebellar GCs. This is important because over forty years ago, Marr speculated that GCs should encode information through sparse activity. Previously, we obtained results using GCaMP3 that called Marr’s speculation into doubt in awake animals (Ozden *et al.*, 2012, *PLoS ONE* 7(8):e42650). The use of Fast-GCaMP6f should let us deconvolve GC signals to extract spike information and clarify this issue quantitatively.

A remaining challenge is improving the on-response to calcium, which is currently in the tens of milliseconds. This design problem requires speeding up the kinetics of calmodulin activation. Based on our molecular model, this may be possible by “fusing” the C-lobe by replacing some  $\text{Ca}^{2+}$ -binding acids with bases – or even by inserting cysteine pairs to generate disulfide bonds.

**REFERENCE:** Sun X.R., Badura A., Pacheco D.A., Lynch L.A., Schneider E.R., Taylor M.P., Hogue I.B., Enquist L.W., Murthy M., Wang S.S.-H. (2013) Fast GCaMPs for improved tracking of neuronal activity. *Nature Communications* 4:2170. doi: 10.1038/ncomms3170.