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 response kinetics than OGB-1, whose τ_r is ~1 ms to a step increase in calcium and τ_o is ~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 using two-photon laser scanning microscopy to characterize our most effective variants, which include those that detect single action potentials and/or firing rate modulations in the 1-100 Hz range.

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 components 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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