SENSORS AND PROBES

# Calcium sensors reach new heights

**A handful of new 'GCaMPs' with improved properties for imaging neuronal activity are now available.**

Neural activity—in particular, the transmission of electrical bursts or 'action potentials' by neurons—causes changes in intracellular free calcium concentration. Using sensors to image the changes in cellular calcium in individual neurons has become a popular way of monitoring neural activity and is applied at all scales, from large neuronal populations to intracellular compartments within a cell. Activity-related changes in neuron calcium concentration lag behind the fast changes in membrane voltage that occur during the firing of action potentials but are nonetheless fast enough that they pose challenges for the development of sensors. The changes in calicum that occur in these conditions are also relatively small. Thus, for neuroscience applications, sensors need to have high sensitivity and respond rapidly to rises and decays in cellular calcium.

Among the most popular genetically encoded calcium sensors are the GCaMPs, which contain the fluorescent protein GFP bound to the calcium-binding domain from calmodulin. As a result of calcium binding, a conformational change in the protein results in changes in the fluorescence of GFP. In the last few years, several research teams have been working to improve the performance of GCaMPs by using random or targeted mutagenesis. These different efforts have recently yielded a collection of new GCaMP sensors with improved sensitivity, faster kinetics or both compared to the popular GCaMP3 and GCaMP5.

A team of scientists from the Janelia Farm Research Campus and their collaborators screened hundreds of GCaMP variants, obtained by targeted mutagenesis, in cultured rat neurons and identified several mutants with improved properties (Chen *et al.*, 2013). One such mutant, GCaMP6s (with 's' for 'slow') had much higher sensitivity than previous GCaMP variants and could resolve individual spikes within bursts of action potentials in the mouse visual cortex. The sensitivity of this sensor was high enough to detect changes in calcium concentration in the small subcellular regions where communication between two neurons typically happens (called dendritic spines) in the living animals. A different variant, GCaMP6f (with 'f' for 'fast') also showed improvements in sensitivity relative to GCaMP3 and GCaMP5 (but less than GCaMP6s) but, in addition, displayed improved kinetics relative to these prior versions. The team also described a third GCaMP6 variant with intermediate properties in terms of sensitivity and kinetics between the fast and slow versions—which they called GCaMP6m.

In addition to these efforts, two other recent reports describe improved GCaMP variants. A group led by Samuel Wang at Princeton University reports a collection of variants, called 'fast-GCaMPs', that showed faster on and off kinetics than GCaMP3 and GCaMP5, with similar sensitivity. The group applied the sensors to track the activity of auditory neurons in response to courtship songs in *Drosophila* (Sun *et al.*, 2013). And Masamichi Ohkura and colleagues from the Brain Science Institute at Saitama University developed GCaMP variants exhibiting higher sensitivity than GCaMP3 (GCaMP5 was not directly compared) and then targeted the sensor to dendritic spines by fusing it with the cytoskeletal protein actin to monitor local changes in calcium concentration (Ohkura *et al.*, 2012).

The good news for neuroscientists is that they now have genetically encoded calcium sensors that are much better than they used to be a few years ago and that surpass the properties of calcium dyes in both sensitivity and kinetics. Because the strategies followed for the optimization of these sensors were all slightly different and have yielded different mutations, it may be possible to combine such mutations to generate even better GCaMPs. The bad news is that neuroscientists will continue to face the dilemma of choosing which sensor better suits their needs. But choice, in this case, is probably not such a bad thing.

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RESEARCH PAPERS

Chen, T.-W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295–300 (2013).

Ohkura, M. *et al.* Genetically encoded green fluorescent $Ca^{2+}$ indicators with improved detectability for neuronal $Ca^{2+}$ signals. *PLoS One* **7**, e51286 (2012).

Sun, X.R. *et al.* Fast GCaMPs for improved tracking of neuronal activity. *Nat. Commun.* **4**, 2170 (2013).