SENSORS AND PROBES

A snapshot of active neurons

A new calcium sensor allows the permanent labeling of neurons that are active at defined periods of time.

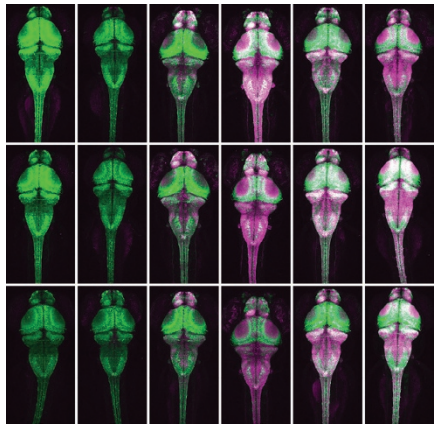
Neural activity can be monitored in different ways. Calcium sensors provide a way to examine neural responses in real time, whereas detecting expression of immediate-early genes can be used to analyze activity after the fact. Eric Schreiter and his colleagues at Janelia Farm Research Campus recently developed another tool to visualize active neurons. This tool, CaMPARI (calcium-modulated photoactivatable ratiometric integrator) combines the advantages of calcium sensors and detection of immediate-early gene expression by providing a stable mark of elevated calcium levels in genetically defined neuronal populations.

CaMPARI converts from its green to its red state only when it is illuminated with violet light and when, at the same time, calcium levels are elevated. To achieve these properties, Schreiter says that it wasn't too much of a conceptual stretch to combine genetically encoded calcium sensing and photoconversion. He thought that "we could probably take one of the photoconvertible fluorescent proteins and instead of modulating the fluorescence intensity like we do with [the genetically encoded calcium indicator] GCaMP, we could modulate the efficiency of that photoconversion process."

Calcium indicators such as the GCaMP variants basically consist of a calcium-binding domain and a circularly permuted fluorescent protein. Schreiter and his colleagues applied the same design principles to the photoconvertible protein mEos2 to create CaMPARI. As the team wasn't certain whether it would mechanistically be the same to modulate photoconversion efficiency and fluorescence intensity, they generated libraries of circular permutations at locations all around the 11-strand β-barrel. "And as it turned out, β-strand number 7, which is the one that is permuted in all the other sensors, seems to be magical and turned out to be the most useful circular permutation site in our sensor as well," says Schreiter.

The properties of CaMPARI make it suitable for experiments in cell culture and *in vivo* in a variety of different systems. CaMPARI is about half as bright as its parent fluorescent protein mEos2, but the fluorescence is fairly stable even after fixation. When calcium levels are elevated, the protein photoconverts 20 times faster than in the absence of calcium. And the sensor does not seem to be toxic or exhibit any long-term adverse effects. Both green and red states can be imaged using two-photon microscopy. However, "it seems that most of these proteins don't photoconvert very efficiently under two-photon excitation," mentions Schreiter; photoconversion is most efficient under one-photon conditions.

To demonstrate the suitability of CaMPARI as a marker for neural activity *in vivo*, Schreiter and his team expressed the sensor in zebrafish, fruit flies and mice. They subjected freely behaving zebrafish expressing CaMPARI pan-neuronally to a variety of different stimuli such as heat, cold, turbulent water or seizure-inducing reagents and illuminated the fish with violet light at the same time to induce CaMPARI photoconversion. As expected, the researchers observed different photoconversion patterns in the fish brains, which is consistent with different neurons being activated by these different stimuli. Similarly, in mice expressing CaMPARI in the visual cortex, neurons that were sensitive to a particular orientation of a moving stimulus were specifically labeled when the mice were exposed to this type of stimulus. Finally, Schreiter and his colleagues demonstrated the use of CaMPARI as an equivalent to trans-synaptic markers known in vertebrates. They expressed CaMPARI in all neurons in the fly brain and exposed the flies to a particular odor while applying violet light. This allowed them to trace the pathways of information flow evoked by olfactory stimulation.



Larval zebrafish expressing CaMPARI in all neurons were exposed to different stimuli. Image reproduced from Fosque *et al.*, AAAS.

Schreiter can imagine a variety of other applications for the CaMPARI sensor or further improved variants, such as functional connectomics, transcriptional profiling based on neural activity, or the comprehensive mapping of synaptic inputs to a neuron, potentially with array tomography. "There are probably a bunch of applications out there that we haven't even thought of," says Schreiter.

Nina Vogt

RESEARCH PAPERS
Fosque, B.F. *et al.* Labeling of active neural circuits *in vivo* with designed calcium integrators. *Science* **347**, 755–760 (2015).