SENSORS AND PROBES

Neuronal activity in multicolor

Multicolor calcium indicators pave the way to a better understanding of information processing in the brain.

Genetically encoded calcium indicators (GECIs) are used to measure intracellular calcium (Ca^{2+}) dynamics as a readout of neuronal activity. Most common GECIs are fluorescent proteins that increase their signal after binding to Ca^{2+} ions. They are based on GFP, the Ca^{2+}-binding protein calmodulin (CaM), and a Ca^{2+}/CaM-sensing peptide. Although the performance of green calcium indicators has been improved enough to resolve single action potentials (APs), most GECIs are limited in terms of sensitivity and two-photon imaging depth, and their kinetics is too slow to image the activity of fast-spiking neurons. Also, AP-resolvable GECIs are only available in green and red.

Haruhiko Bito and coworkers at the University of Tokyo have engineered a suite of GECIs of different colors (XCaMPs) with superior spatial and temporal resolution and a linear response to Ca^{2+}, which enable the detection of higher-frequency APs in neuronal somata and dendrites in vivo. The researchers combined G-CaMP4.1, a green GECI with high signal amplitude, with a mutant higher-affinity Ca^{2+}/CaM-sensing domain from the neuronal protein CaMKK. Then, by mutating the chromophore region, they generate green, red, yellow and blue variants. They used these multicolor GECIs to record high-frequency spiking in parvalbumin-positive interneurons and to perform multiplex spectral imaging in four neuron groups in freely moving mice. XCaMPs also allow dual-color paired recordings across synapses in vivo and two-photon hippocampal imaging up to 1.1 mm deep in the mouse brain.

This expanded GECI color palette provides greater flexibility for neurophysiology and optogenetic experiments, and it comes alongside other recent advances in GECI development. A notable example is the new set of jGCaMP7 sensors engineered by Dana et al. at Janelia Research Campus, which come in a range of kinetics properties and basal fluorescence levels tailored to fit different applications. These improved sensors will help further our understanding of information processing in brain circuits in vivo, at both the single-neuron and the neuronal-ensemble level.

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Research papers
Inoue, M. et al. Rational engineering of XCaMPs, a multicolor GECI suite for in vivo imaging of complex brain circuit dynamics. *Cell* **177**, 1346–1360 (2019).
Dana, H. et al. High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. *Nat. Methods* https://doi.org/10.1038/s41592-019-0435-6 (2019).