NEUROSCIENCE

Sensing calcium in red

Red-shifted calcium sensors are advantageous for deep-tissue imaging, dual-color applications and combining with optogenetic tools.

Genetically encoded calcium indicators such as the green-fluorescent GCaMP6 sensors have revolutionized the analysis of neural activity in model organisms, as they allow noninvasive or minimally invasive recordings. Red-fluorescent sensors would have advantages for *in vivo* applications, especially in deeper tissues, owing to reduced scattering, but the performance of red-fluorescent indicators has lagged behind.

Hod Dana and his colleagues from the Janelia Research Campus in Ashburn, Virginia, developed red-fluorescent calcium sensors that approach the GCaMP6 state of the art. As a first step, the researchers randomly mutated sequences in the existing RCaMP1h and R-GECO1 sensors, on the basis of their previous experience with engineering improved green-fluorescent calcium sensors. Next, the researchers combined beneficial mutations to arrive at jRCaMP1a, jRCaMP1b and jRGECO1a.

In neuronal cell cultures, jRGECO1a displayed the largest change in fluorescence per action potential of the trio, whereas jRCaMP1b had the largest dynamic range and could report up to 160 action potentials without reaching saturation. In the mouse visual cortex, the brightness of jRCaMP1a allowed it to be imaged up to layer 6. jRGECO1a signals were comparable to GCaMP6f signals and reported visual stimuli more faithfully than jRCaMP1, owing to faster kinetics. jRGECO1a was also best in class in *Drosophila* and even outperformed the GCaMP6 sensors in zebrafish neurons, whereas jRCaMP1b was best suited for reporting calcium signals in *Caenorhabditis elegans* neurons.

The performance of the newly developed red-fluorescent calcium indicators should make them a good choice for a variety of applications. Some caution may be called for, however. jRGECO1a tends to accumulate in lysosomes *in vivo*. Furthermore, jRGECO1a photoswitches when illuminated with blue light, similar to other fluorescent proteins based on the same scaffold. Finally, the researchers noticed that all three red-fluorescent indicators are present in two different species, one of which is not responsive to calcium changes. Overcoming these challenges may require additional protein engineering. Nevertheless, it will be interesting to see these calcium sensors in action.

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RESEARCH PAPERS

Dana, H. *et al*. Sensitive red protein calcium indicators for imaging neural activity. *eLife* **5**, e12727 (2016).