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Genetically encoded calcium indicators taste the rainbow

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Research Highlight: *Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Frontiers in Molecular Neuroscience. Akerboom et al, March 2013, Volume 6, Article 2.*

Abstract : A new range of genetically encoded calcium indicators have been developed to allow two color imaging and more sensitive cellular imaging. The authors of this paper go on to show the use of these genetically encoded indicators in living animals, including worms, zebrafish and drosophila under physiological conditions. The complexity of measurements now possible opens the door to interesting new in vivo as well as in vitro experimentation.

Keywords : Indicators, Genetically encoded indicators, optical sensors, GCaMP

In neurons, action potentials open calcium channels, where the rapid influx of calcium stimulates the fusion of neurotransmitter containing vesicles. The invention of fluorescent dyes, which change their intensity of photon emission upon calcium binding, created a watershed of fundamental studies exploring calcium signaling and cell physiology by neurobiologists (amongst others). However, loading dye into a living animal is problematic for a host of reasons. To overcome this problem many attempts were made to develop a bright and photostable genetically encoded calcium indicator. A seminal paper[1] was published in 2008 by a group at Janelia Farms wherein improvements were made to a previously published genetically encoded calcium indicator (GECI) protein called GCaMP[2], a protein chimera containing a circularly permutated GFP (N and C termini fused with a new opening in protein) fused to a calcium binding protein (calmodulin) and a myosin light chain (M13). The permutated GFP increased its photon emission when exposed to calcium due to structural shifts in calmodulin and M13 interactions that shield the chromophore. This modified GCaMP (GCaMP3) protein was both bright and stable and was able to detect the physiological influx of calcium when expressed freely in the cytosol or tagged to proteins on organelles within the neurons of live organisms.

What more could physiologists want? Well, to be honest, something not green! Green fluorescence is excited by blue light, which has three disadvantages for use *in vivo*. First, blue light excites a host of autofluorescence, thusgreatly decreasing the levels of signal to noise. Second, the short wavelength of blue light is more easily scattered by tissue. Third, a widely used optogenetic tool channelrhodopsin-2 (ChR2) , is activated by the same blue light which excites GCaMP, making a combination of the two impossible.

The same group that created GCaMP3, Akerboom *et al* , have conducted a thorough study of a bevy of new spectrally shifted GCaMPs. They report the design of three spectral “XCaMPs” (blue, cyan, and yellow) by rational mutagenesis in ways similar to GFP

variants. Most importantly, they have designed three new red variants of GCaMP dubbed "RCaMPs" with the newly discovered circularly permutated fluorophore mRuby. This work, following on the heels of another recently designed red-shifted XCaMP named R-GECO1 (found by permutating mApple and designed by Zhao *et al)* was the first to break the color barrier for GECIs. Akerboom's study is chock full of important diagnostic tests, which were exhaustively performed to study spectral absorbance, calcium affinity, pH-dependence and the all-important relationship between change in fluorescent emission and calcium concentration. They also performed tests to compare one-photon and two-photon imaging (especially important for *in vivo* imaging) and studied bleaching and quantum efficiency characteristics under these excitation conditions. The authors also demonstrated the use of RCaMPs in living animals, including worms, zebrafish and drosophila under physiological regimes of stimulation. They also show proof of principle experiments labeling two types of cells in a single culture (neurons and astrocytes). Finally the authors also demonstrate the successful use of RCaMPs with ChR2 without spectral overlap.

These RCaMPs are bright and photostable and will finally allow simultaneous imaging with green fluorophore or blue-light activated proteins. An important and interesting finding is that R-GECO1, although having the highest calcium sensitivity and largest change in fluorescence when binding to calcium, is prone to photo-switching (i.e. no longer fluorescent in the red spectrum when illuminated by blue light) as was previously reported for mApple. As a result, two color imaging (green and red fluorescence) is most safe using these newly discovered RCaMPs. For any researchers in neurobiology or those studying calcium kinetics in other cell types, these findings will be of great use. Best of all, these proteins are already available on addgene (www.addgene.org).

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