ALS SCIENCE HIGHLIGHT

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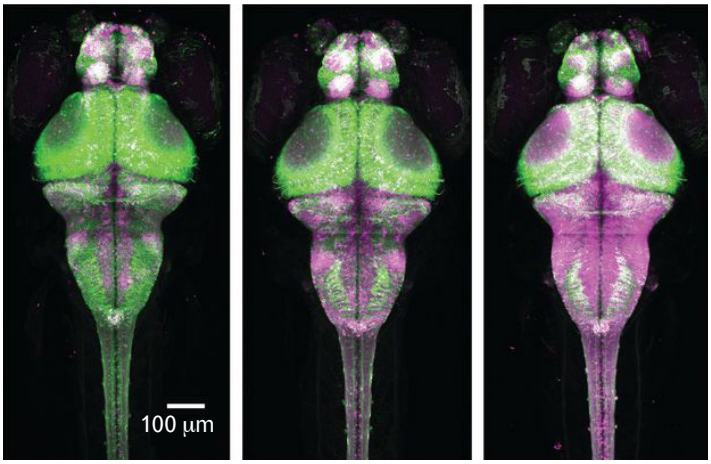
A Designed Protein Maps Brain Activity

A team of scientists from the Howard Hughes Medical Institute's Janelia Research Campus designed and validated via x-ray crystallographic studies a fluorescent protein (CaMPARI) that allows the permanent marking of active brain cells. The protein was then used to study live changes via fluorescence in the active nerve cells in brains of fruit flies, zebrafish, and mice.

In theory, one can monitor the levels of calcium ions in brain cells to see which circuits are lighting up and when. But the signals are transient, so that after the activity is over (sometimes as fast as microseconds) the ion levels change again. Closely related previous studies used dyes or proteins that change color when they bind to calcium, with the color change being monitored with a microscope. This approach is an excellent step in imaging brain circuits, but a limitation is that the microscope has to be pointing at the area of activity when it changes, otherwise it will miss the event. Another limitation is that the brain being monitored has to be still, so that the microscope can focus on the area where the calcium levels are changing.

The breakthrough made by this research team was to combine two different protein "technologies." The first technology is a protein that permanently changes color when it binds to calcium. Using this protein, neural activity (as measured by calcium concentration) can be stored in the color of the protein, like a switch that has flipped and only has to be looked at later to determine whether it is on or off. The other key aspect of the breakthrough was to ensure that the change in color only takes place when the calcium-bound protein is exposed to violet light. This meant that the scientists could shine violet light on the animal while it was engaged in a particular activity (for instance, when a fruit fly was exposed to a particular odor), and the brain circuits firing during that particular activity are then effectively captured for later study. The designer protein is called CaMPARI (calcium-modulated photoactivatable ratiometric integrator).

The part of CaMPARI that binds to calcium is called calmodulin, and the part that fluoresces in different colors in response to violet light is called Eos. Engineering CaMPARI required combining these two components in thousands of different variations until the product gave a high enough response to both calcium concentration and light. The researchers in this study then used it to show brain activity in zebrafish as a response to water temperature and to trace the brain-circuit response of fruit flies to different odors.



CaMPARI expression in zebrafish larvae under various conditions.

Publication about this research: B.F. Fosque, Y. Sun, H. Dana, C.-T. Yang, T. Ohyama, M.R. Tadross, R. Patel, M. Zlatic, D.S. Kim, M.B. Ahrens, V. Jayaraman, L.L. Looger, and E.R. Schreiter, "Labeling of active neural circuits in vivo with designed calcium integrators," *Science* **347**, 755 (2015).

Research conducted by: B.F. Fosque, Y. Sun, H. Dana, C.-T. Yang, T. Ohyama, M.R. Tadross, R. Patel, M. Zlatic, D.S. Kim, M.B. Ahrens, V. Jayaraman, L.L. Looger, and E.R. Schreiter (Howard Hughes Medical Institute).

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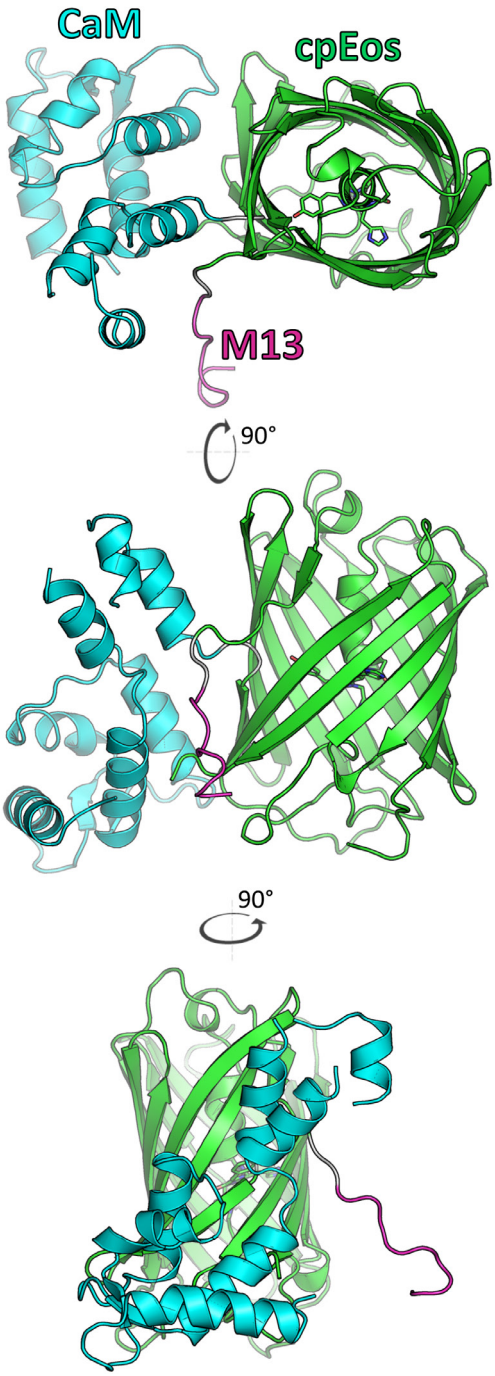
The Neural Basis of Behavior

Signals in our brains are propagated with voltage and salts. As you read these words, your brain is sending voltage spikes along your brain cells (neurons), which then cause specialized pores (ion-channel proteins) to open or close, causing ions such as sodium or calcium to flood in or out of the neuron. This in turn causes neurotransmitters to be released and detected by other neurons. The transmission of neurotransmitters is what underlies the basic mechanism underlying all the processes of our brain: thinking, memory making, and commands that control the rest of our body.

How can we possibly monitor this complex interplay of ions and proteins to determine how it leads to brain activity? This has long been a goal of neurologists: to track the neural paths in the brain that correspond to specific activities. Mapping the "brain circuits" involved in the huge range of animal activities (such as walking, eating, and memory retrieval), as well as what parts of the brain are enlisted to store and retrieve memories or experience emotions, helps us not just understand ourselves, but also leads to an understanding of what causes the brain to malfunction, such as in autism, diseases, and mental disorders.

How does protein crystallography play a role in all of this? As in many cases, crystallography offers a way to visualize the underlying structure of a molecule in order to both validate a design and further improve on a design. In the case of CaMPARI, the scientists needed to know the structure of the new tool that they had designed in order to understand exactly how it worked. They brought CaMPARI to Beamline 8.2.2 at the ALS to solve its molecular structure. The results show exactly how Eos and calmodulin are connected and how the engineered mutations in each allow the combined proteins to function together as one unit.

CaMPARI is an outstanding new tool for mapping brain activity, but it could be made even more sensitive and versatile. The researchers in this study are continuing to improve it, but they are also releasing all information about CaMPARI, as well as the DNA needed to produce it and the zebrafish expressing CaMPARI, to make it available for other scientists. In this way, other scientists can not only use it, but improve on it as well. After all, the more brains working on brain tools, the better.



Crystal structure of calcium-free CaMPARI. Three orthogonal views of the same structure are shown as a cartoon, colored by domain.