SENSORS AND PROBES

All-in-one optogenetics

Scientists reverse engineer fluorescent proteins for light-mediated control.

Optogenetics is a young discipline that is coming on strong in fields such as neuroscience and protein signaling. It refers to the use of light-sensitive proteins to control cellular processes in living cells and organisms. Optogenetic tools can also be used to sense biological processes. Each of these applications has been performed with separate protein tools—until now.

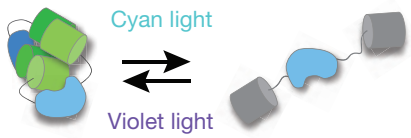
Michael Lin, at Stanford University, and his colleagues have adapted a fluorescent protein (FP) to act as a light switch for controlling protein interactions, creating a protein tool that can both mediate biological function and report its own activity.

"If we could get fluorescent proteins to work as light-controlled switches, they would come with distinct advantages," says Lin about the reasons for embarking on the project. One such advantage is that FPs have built-in chromophores and thus do not rely on an exogenous chromophore supply as many light-controlled systems do. FPs are also relatively easy to engineer, and a nice color selection is available to choose from.

Some FPs can be switched on or off in response to specific wavelengths of light. This process occurs through light-induced conformational changes in the protein. In a way, FPs have structural and functional features analogous to those found in natural light-regulated signaling molecules, thought Lin, so he and his team set out to look for FPs with promising properties for their experiments. "What we wanted was something that would be bound in the dark and that would unbind in response to light," he says. They got it right on their first try with the FP Dronpa.

The team found that a mutant form of Dronpa was brightly fluorescent in its tetrameric form in the dark, but upon cyan light illumination it dissociated into a nonfluorescent monomer. The nonfluorescent state could be reversed using ultraviolet light, too.

From that point on, it was easy for Lin and his colleagues to see Dronpa's potential. All they had to do was fuse Dronpa domains to each of the ends of the protein they wanted to control. In the dark, the domains would associate, 'crunching' the middle protein and rendering it inactive. Light would then release the domains from this embrace and 'uncage' the protein.



Controlling the activity of a protein (blue) using light and Dronpa (cylinders). Image courtesy of M. Lin.

Lin and his colleagues first used this approach to engineer a light-controlled version of the guanine nucleotide exchange factor protein intersectin, which produces membrane extensions in cells. Cells expressing the engineered protein were locally illuminated, and the researchers observed filopodia extending under the spot light. Because Dronpa dims when its domains are dissociated, the same tool served to report intersectin's active state as well.

The researchers also used the approach to engineer a light-activatable protease, a class of enzymes for which light activation hadn't yet been achieved.

Lin and his colleagues are interested in applying their dual optogenetic tool in neurons, because in these highly branched cells much of the signaling is spatially controlled. *In vivo* application should also be straightforward using two-photon illumination, Lin believes.

The team is now running down their 'to do' list. They first want to make the proteins more effectively turn on and off in response to light (currently they estimate that about 50% of the proteins get turned on after a 30-s light pulse). They also want to develop similar tools that respond to other colors of light and to tackle harder-to-engineer proteins such as kinases.

Lin thinks that FPs haven't been used in this manner before because of a problem of perception: "Given their success as imaging agents, people haven't thought of them any other way." FPs were already fascinating proteins, and these findings make them even more so.

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RESEARCH PAPERS

Zhou X.X., *et al.* Optical control of protein activity by fluorescent protein domains. *Science* **338**, 810–814 (2012).