

The Scientist

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By Amber Dance

Guiding light

How to manipulate cellular events with the right light sensing molecule and a flash of light.

Using light-producing molecules to observe cellular events is standard fare in many a lab, but it's only recently that scientists have begun to harness the power of light to manipulate biological systems experimentally. By rigging a cell or protein with a light-sensing molecule, it's now possible to alter cellular biochemistry or neural action potentials with the flash of a laser.

Tweaking a cell or organism with gene knockouts, small molecule inhibitors, or RNA interference leaves it time to compensate, potentially muddying results. But a light signal takes effect instantaneously, providing unprecedented temporal and spatial precision. Just as green fluorescent protein revolutionized cell biology by tracking light output, "these genetically encoded photoswitches will raise perturbation to that same level," says Michael Rosen of the University of Texas Southwestern Medical Center in Dallas.

But photoswitches require finesse: to adapt nature's light sensors for your own use, you'll likely have to optimize factors such as protein-protein interactions, intramolecular linkers, and gene expression levels. Activating a few cells or part of a single cell requires skill in surgery or microscopy. And different molecules work best for different types of experiments.

Here, *The Scientist* profiles researchers using four types of light-sensitive switches to give you the lowdown on how to flip them.



Light Sensor: Channelrhodopsin



Channelrhodopsin, a cation channel from green algae, opens up in response to blue light. In the alga, it helps the cell orient the direction of its swimming. In the lab, scientists have planted channelrhodopsin in neurons, where the opening of the gate depolarizes the nerves, causing them to fire.

USER: Patrice Guyenet, Professor of Pharmacology, University of Virginia, Charlottesville

PROJECT: Guyenet and his colleagues had put together a strong case linking a specific set of neurons, the retrotrapezoid nucleus, to regulation of breathing. But they lacked a critical proof: “We needed to demonstrate that these neurons, when you activate them, do in fact raise breathing,” Guyenet says.

The researchers turned to a light switch developed by Karl Deisseroth of Stanford University and others. They hooked the channelrhodopsin gene to a promoter specific for these cells and used a lentiviral vector to deliver the transgene into the brains of rats. Then, they implanted a fiber optic to deliver light with one end next to the cells of interest in the brain and the other attached to a laser diode outside the head (*J Neurosci*, 29:5806-19, 2009).

To activate the retrotrapezoid nucleus, they sent blue light down the fiber optic. The neurons expressing channelrhodopsin—a few hundred of them—responded by firing. “And lo and behold, we did find robust activation of breathing,” Guyenet says.

CONSIDERATIONS: It is essential, Guyenet says, to perform basic electrophysiology experiments to confirm that the intended cells are indeed firing in response to light. And he cautions that artificially activating one set of neurons in no way mimics a natural signal: “These cells are not stimulated in a vacuum.”

PROS:

- Light input does not interfere with electrophysiological readouts
- Can be combined with other opsins that respond to different wavelengths
- Changing the frequency of laser pulses modulates rate of nerve firing

CONS:

- Neurons only
- Channel is leaky and nonselective
- Requires a strong promoter

MATERIALS: Deisseroth shares protocols and clones at www.optogenetics.org,

and Ed Boyden of the Massachusetts Institute of Technology in Cambridge, another developer of the technology, does the same at www.syntheticneurobiology.org. The main equipment cost is the laser: “If you want a good one, you have to fork out about \$10,000,” Guyenet says.

Light Sensor: Caged ATP



Caged ATP is conjugated to a molecule that renders it unavailable, but ultraviolet light unlocks the cage. Researchers have paired this handy molecule with an ATP-gated ion-channel, expressed in neurons, that allows cations to flow into the cell: freeing the ATP opens the channel, creating an action potential that causes the neuron to fire.

USER: Gero Miesenböck, Waynflete Professor of Physiology, University of Oxford, UK

PROJECT: Miesenböck and colleagues wanted to make light-controlled *Drosophila*. They first targeted the two nerves of the simple, well-understood giant fiber system. Normally, when these neurons fire, the animal jumps up and flies away.

The researchers engineered the flies to express an ATP-activated rat pain receptor, P2X2, in the two giant fiber neurons (*Cell*, 121:141-52, 2005). ATP, provided extracellularly, would bind the receptor, open the channel, and activate the nerves.

The scientists injected caged ATP into the flies’ heads. When the insects were exposed to the appropriate wavelength—conveniently, flies are sufficiently light-permeable that the external UV laser reached their nerves—the ATP was released to open the floodgates and activate the giant fiber nerves. As a result, the flies jumped and flapped their wings.

CONSIDERATIONS: This system is stronger than other methods in *Drosophila*, Miesenböck says: “You cannot get a fly to jump around by putting channelrhodopsin in [its] place.” However, the nature of the sensor means the effect is not reversible—once unlocked, the cage will not close again—and a sufficient supply of caged ATP is required. Scaling up to larger animals could be tricky. “If you wanted to fill the skull of a mouse with a light-sensitive chemical, you’d need quite a bit of it,” Miesenböck says. The researchers also had to make sure the insects were not simply responding to a visual cue—which they did by genetically blinding the flies, or, in a crude yet effective experiment, by removing their heads.

PROS:

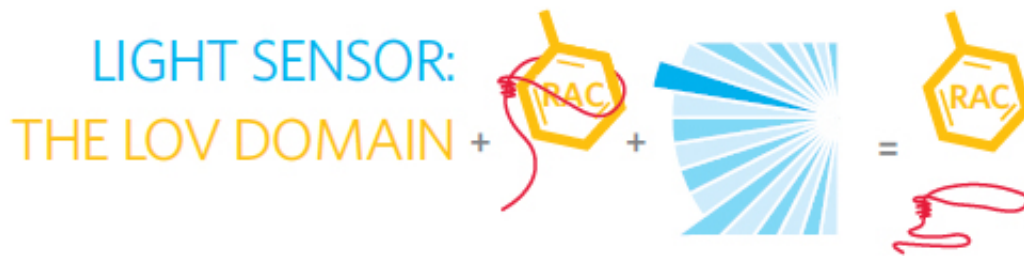
- Stronger than channelrhodopsin in invertebrates
- Can use 2-photon excitation to activate cells with 1-micron resolution

CONS:

- Neurons only
- Not reversible
- Requires exogenous molecule

MATERIALS: DMNPE-caged ATP is available from Invitrogen (\$181/5 mg). Targeting light to specific cells, say, in a brain slice under a microscope, requires a focused laser, but the optics for whole-fly experiments are much simpler, Miesenböck says. “Depending on what your readout is, you can set this up in an afternoon.”

Light Sensor: The LOV Domain



The LOV domain, found within the light-sensitive plant protein phototropin as well as some bacterial proteins, responds to light, oxygen, or voltage (hence the name). Researchers can link LOV to other proteins to block their activity with a reversible, light-controlled interaction.

USERS: Klaus Hahn, Distinguished Professor of Pharmacology, and Yi Wu, Research Assistant Professor, University of North Carolina, Chapel Hill

PROJECT: First, the researchers sought to make a light-activated protein. They chose Rac, a GTPase that activates cell motility. Wu attached Rac to the LOV domain from oat phototropin, such that the LOV domain sterically blocked Rac's activity. With a flash of blue light under the microscope, the LOV domain swung away from Rac, freeing it to function (*Nature*, 3:104-08, 2009).

Wu could then turn on Rac by shining a blue laser at different parts of the cell. When he activated Rac on one edge of the cell, the cell crawled in that direction.

Next, the researchers used the system to probe Rac's interaction with another GTPase, Rho. Both affect cell motility, via cytoskeleton rearrangements, but biochemical studies resulted in conflicting evidence on the precise relationship between the two, Hahn says. They designed an experiment to determine whether Rac would activate or inhibit Rho by combining the photo-activated Rac with a biosensor for Rho activity. Using the laser to turn on Rac, they confirmed that Rho turned off in the same area.

CONSIDERATIONS: The LOV domain has no precise "off" switch; once the light is gone, the conformation returns to the dark state over approximately a minute, although fast- and slow-relaxing mutants are available. Finding the right linker between the LOV domain and protein "could be challenging and involves a lot of structural knowledge," Wu says. The researchers estimate they tried 20 different versions of the fusion protein.

PROS:

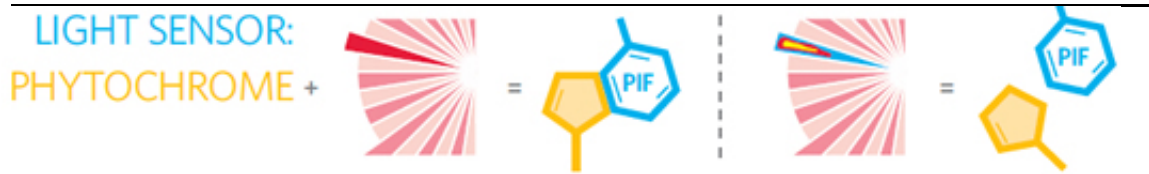
- Can target activation to portions of cells
- Flavin chromophore, a required cofactor, is near-ubiquitous in living organisms
- Steric inhibition should be applicable to many proteins

CONS:

- No precise off switch
- Linker requires optimization

MATERIALS: “You can do it with a mercury arc lamp and an aperture,” Hahn says, “but really it’s much easier if you have a laser.” The researchers used a photobleaching set-up to target the light to specific parts of the cell (MAG Biosystems FRAP-3D, Photometrics, \$80,000-\$100,000).

Light Sensor: Phytochrome



Phytochrome, a light-sensitive protein found in plants and bacteria, binds its partner PIF (phytochrome interaction factor) upon exposure to red light. Infrared light breaks their association. In plants, the bound pair translocate to the nucleus to activate transcription.

USERS: Wendell Lim, Professor of Cellular and Molecular Pharmacology and Biochemistry and Biophysics, and Anselm Levskaya, graduate student in biophysics, University of California, San Francisco

PROJECT: The researchers theorized the phytochrome-PIF pair could bring any two proteins together, but the interaction was weak and slow to reverse. Levskaya tried 30 or 40 pairings—he lost count—to find the optimal strength of binding and ideal unbinding kinetics.

Then, the researchers modified both phytochrome and PIF. They swapped phytochrome’s nuclear localization tag for a cell membrane localization sequence, so that upon PIF binding, both members of the pair would move to the plasma membrane. Levskaya then linked PIF to Tiam, which activates Rac to initiate cell crawling (*Nature*, 461:997-1001, 2009).

Under the glow of a red laser, the partners found each other and headed for the membrane, where Tiam induced the cell to send out lamellipodia. By aiming the red light at part of the cell, while the rest was flooded with infrared, Levskaya could induce single processes up to 30 microns long.

CONSIDERATIONS: “If there is any universal signaling currency in cells, it is protein-protein interaction,” Levskaya says. Phytochrome and PIF could be the currency exchange allowing scientists to reversibly force any two proteins together. The system requires both proteins as well as a chromophore, phycocyanobilin, not naturally present in most cells. But phycocyanobilin is readily available: “You can go down to your health-food store, buy a big bottle of spirulina, and it turns out to be a fairly simple extraction procedure,” says Rosen, who also works with phytochrome.

PROS:

- Precise on and off switches
- Can target stimulation to part of a cell
- Phytochrome-PIF interaction already optimized

CONS:

- Three components required
- Finding the right expression levels could be tricky

MATERIALS: To combine red and infrared signals in one microscopic field, you’ll need a set-up that can digitally mix the two wavelengths, not unlike the chip in a digital projector. Photonic Instruments’ Mosaic digital illumination system sells for \$50,000-\$90,000. Or, Levskaya says, “If you have a little bit of savvy with hacking things together, you can build your own for a nominal fee” (\$5,000-\$10,000).