

Traditional methods for controlling biological signals in cells are a sledgehammer: they are global, slow, and often non-specific. The authors of this paper describe their new technique to generate local, fast, and targeted cell signaling in live cells that are genetically altered to have light-sensitive proteins. They engineered a cellular perturbation system applicable to many signaling proteins. The main requirement for the candidate signaling protein is to be naturally activated by interactions that re-localize it to the membrane.

Levskaya et al. built this membrane recruitment system using photosensitive proteins named Phytochromes. These proteins from plants detect red and near-infrared light through the photoisomerization of a bound chromophore. This light detection changes the Phytochrome's conformation between a state under red light that binds directly to a phytochrome interacting factor (PIF) and a state under infrared light that doesn't bind to PIF. The scientist added a membrane-localization part to the Phytochrome, and attached a signaling protein to the PIF to complete their system. A cell illuminated with infrared light under the microscope will have inactive, free-floating, PIF-attached signaling proteins. When the scientist points a red laser in the phytochrome-rich membrane, the PIF-attached proteins are forced to stay close to the membrane; effectively increasing the activity of the signaling proteins. Turning off the red laser frees the proteins and turns off the cellular signal.

To demonstrate the feasibility of this new technique they focused on the signaling proteins Tiam and intersectin, precursors of the Rho-GTPases Rac1 and Cdc42 that have crucial role in the organization of actin cytoskeleton during cell movement. They performed three main experiments: The first experiment tested if membrane recruitment of a small part of intersectin (ITSN-DH-PH) that regulates Cdc42, was effectively inducing transient increases of local protein activity. They shown images of local enrichment of biosensors responsive to Cdc42 activity in the membrane that disappeared few seconds after turning off the red laser. The second experiment tested if membrane recruitment of a part of Tiam (Tiam DH-PH domain) was sufficient to induce changes in the shape of NIH3T3 cells. They illuminated the whole cell with red light for 20 minutes and immediately after counted the percentage of cells that made new lamellipodia (actin cytoskeletal projection on the mobile edge of the cell). The result was that almost 80% of cells made new lamellipodia under red-light treatment, compared with a 10% of control populations. To make things even more interesting, in a third experiment they pointed a red laser dot on the edge of one cell and gradually moved it outward, slowly extending this red-targeted region from the cell body. They show in movies that they effectively guided the direction followed by the new lamellopodium-- the first reported control of cell movement in real time using light-sensitive proteins!

“Spatiotemporal control of cell signalling using a light-switchable protein interaction.”  
Levskaya et al. Nature 2009.