Scientists created proteins controlled by light

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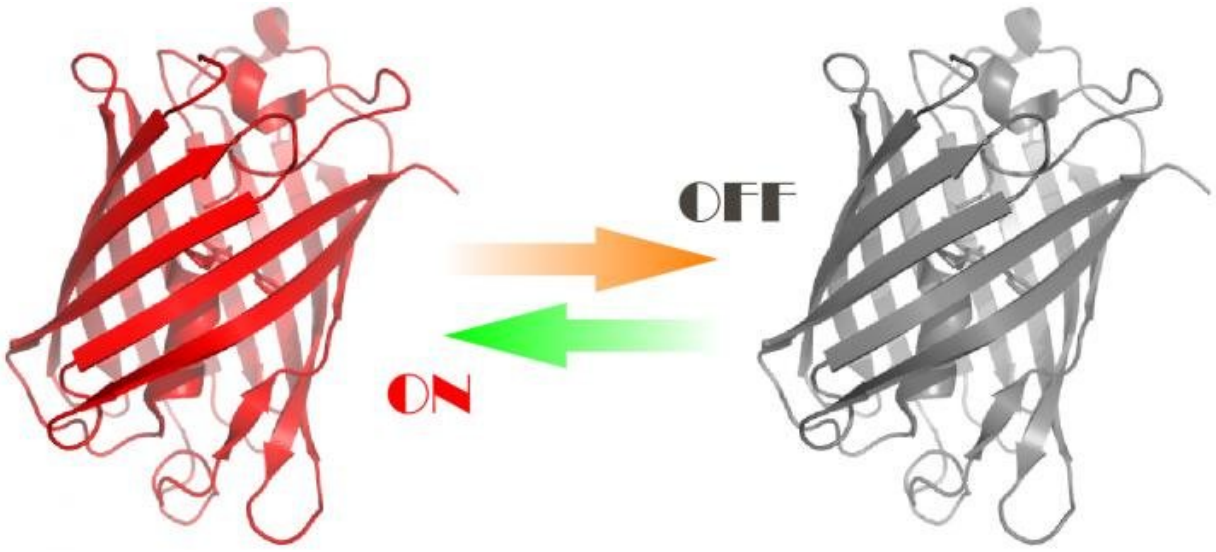

3D model of the developed protein Credit: Aleksandr Mishin

Researchers have developed fluorescent proteins that can be controlled by orange and green light. These proteins will help to study processes in living cells. Results were published in *Nature Methods*.

Fluorescent proteins emit intense visible light with wavelengths ranging from 390 to 700 nm. Natural functions of such proteins are diverse; for instance, some species of jellyfish use green fluorescent spots to attract small organisms that serve as food. Optical properties of certain fluorescent proteins can be controlled with light. For instance, such proteins can be activated and deactivated, and are therefore called

switchable. Switchable fluorescent proteins are widely used in a new group of methods called super-resolution fluorescence microscopy (nanoscopy), which allows imaging of extremely detailed intracellular structures. Currently, scientists usually use blue or violet irradiation for such microscopy, which is highly toxic for cells as it disrupts their normal physiology and can even cause death.

"We were the first to create photoswitchable fluorescent proteins with optical properties that can be controlled using green and orange light rather than blue-violet radiation. The advantage of this is minimal harm to cells. We used new proteins to observe cytoskeleton changes in living cells over time," explained Aleksandr Mishin, Ph.D, senior researcher of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, who headed up the RSF project.

In order to create such fluorescent proteins, the scientists altered them by directed and random mutagenesis via polymerase chain reaction. Then the scientists cloned proteins and selected the best-performing ones using a microscope. The authors analyzed the results of experiments carried out by other biologists and determined how the microenvironment of the chromophore (the aromatic amino acid residue responsible for light absorption in the protein) must be altered to make it capable of photoswitching.

However, the expected outcome does have side effects, including reduced brightness of the protein. The researchers used random mutagenesis to find additional mutations, which compensates for the method's side effects while preserving the target mutation.

The proteins are called reporter proteins, as they act as "spies" within cells. They are attached to other proteins that can then be traced in a living cell. The detailed information obtained thereby can be used in basic science or biomedical research. For instance, tumor cells in cancer

patients exhibit dramatic disturbances of cell mobility and dynamic structural changes in the cytoskeleton, a carcass in the cytoplasm of a living cell. Meanwhile, the investigation of these processes in living cells by nanoscopy is difficult due to the overly intense irradiation of samples, making it necessary to use methods that are less toxic for the organism.

The authors used their development to carry out RESOLFT super-resolution microscopy. The proteins have an important feature: Their photoswitching is very efficient, meaning that fluorescence can be switched on and off in milliseconds. This does not suit all microscopy methods. In some cases, the high speed will only be a nuisance. In RESOLFT, the on-off cycle is repeated many times for adjacent points that are scanned with laser beams. The better the switching time of a fluorescent tag, the faster the complete image can be obtained, as photoswitching at each point requires less time.

"The fluorescent proteins we created will enable super-resolution microscopy without harming the living cell, which opens up opportunities for studying dynamic processes within the cell," Aleksandr Mishin concluded.

More information: Francesca Pennacchietti et al, Fast reversibly photoswitching red fluorescent proteins for live-cell RESOLFT nanoscopy, *Nature Methods* (2018). DOI: 10.1038/s41592-018-0052-9

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