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An optical switch for a motor protein

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Abstract

A ‘photoswitch’ for a motor protein: Incorporation of a photo-cleavable group onto a phosphoserine residue of the regulator of a motor protein allows light-induced activation with spatial and temporal precision inside a living cell.

Keywords

photochemistry; myosin; posttranslational modification; phosphorylation; protein semisynthesis

Essentially every biological process requires protein function to be regulated with temporal and spatial precision. To examine the contribution of a protein to a complex biological process requires methods to inhibit or activate the protein’s function at a selected time and at a particular location inside a cell. Currently, two types of approaches are typically used to study protein functions^[1]. First, proteins are knocked-down in cellular contexts using different methods, including RNA interference. Typically, these perturbations act on timescales of hours or days, and therefore, do not provide the control over protein function on a timescale that matches many cellular processes, which can occur within seconds or minutes. Moreover, it is difficult to control the period over which the protein is inhibited, as it can be difficult to ‘reverse’ the protein’s knockdown. Second, chemical inhibitors can be used to inhibit or activate, often by inhibitor removal or ‘wash-out’, their targets on fast timescales (minutes or even seconds), albeit in some cases with the lack of desired specificity. However, neither of these approaches readily provides spatial control over a protein’s function in cellular contexts.

Photochemistry has the potential to address this limitation. The rationale is that applying a flash of light, focused at a specific region of a cell, could eliminate or generate biologically active molecules locally and fast. An example of such an approach is Chromophore-Assisted Laser Inactivation (CALI, also known as, Fluorophore-assisted laser inactivation, or FALI)^[2]. In CALI, a fluorescent protein is fused to the protein of interest, or a small chromophore-conjugated antibody is introduced into cells to bind the protein of interest. Irradiation at a region of interest with an intense beam of light stimulates the chromophore, which then leads to the production of highly reactive oxygen species (ROS) that, in turn, locally inactivates the protein of interest. As fluorophores can be genetically encoded and does not require chemical synthesis, this approach is accessible to biologists and there are

several nice examples of the use of CALI^[3, 4]. However, concerns remain regarding the specificity in target protein inactivation (rather than inhibition of ‘by-standing’ molecules) and the mechanisms underlying inhibition of function^[5].

Another widely used approach to control protein function using photochemistry involves the design and use of ‘caged’ molecules. Central to this approach is the introduction of a covalent modification, using a photo-cleavable moiety, at a position in the molecule to block its activity. The caged molecule can then be used in cellular contexts and ‘uncaged’ using light. Thus far, a variety of small molecules, such as nucleotides, calcium chelators, amino acids and protein receptor agonists have been used in ‘caged’ forms^[6, 7]. However, the ‘uncaged’ small molecules are likely to diffuse rapidly over micron distances (typical diffusion coefficients are $>10 \mu\text{m}^2/\text{sec}$) and thereby limit the extent of spatial control over protein function.

There are also many examples of the use ‘caged’ proteins to examine function. Direct modifications of a protein’s active site with a photo-cleavable moiety can be used to block function. The photo-cleavable groups can be introduced into proteins by different methods. The simplest approach involves modification of a protein with ‘caging’ groups via reactive functional groups in amino acid side chains. For example, free cysteine residues can be modified by ‘caging’ agents that have an electrophilic moiety. Now, with the development of modern methodologies in protein engineering, such as site-directed unnatural amino acid mutagenesis^[8] and native^[9] or expressed^[10] protein ligation, the caged amino acids can also be directly incorporated into the native protein sequence at a selected site.

While the photoactivation of proteins has been described for a variety of protein classes including kinases, proteases, nucleases, ion channels and antibodies^[6, 7], this strategy can be especially valuable to examine regulation by protein posttranslational modifications (PTMs, e.g. phosphorylation, acetylation and methylation). In the cell, adding or removing PTMs can rapidly change a protein’s structure, its activity, or its interactions with other proteins. When a ‘caging’ group is used to ‘mask’ a PTM, the light-mediated ‘uncaging’ can reveal this PTM, thereby mimicking the fast intracellular changes that can be induced by the PTM.

A recent study by Imperiali and colleagues provides an elegant application of this strategy to study phosphorylation-dependent regulation of protein function^[11]. The study focused on myosin II, an actin-based motor protein essential for many cellular processes, including cell migration and division. Myosin II is regulated by phosphorylation at Ser19 of a binding partner called myosin regulatory light chains (mRLC). Imperiali and co-workers incorporated a 1-(2-nitrophenyl)ethyl (NPE)-caged phosphoserine in this residue by protein semi-synthesis, using native chemical ligation (NCL). The synthetic caged-mRLC cannot activate myosin *in vitro*. But once ‘un-caged’ by UV irradiation at 365 nm, the active protein is released immediately and shows robust activity, as analyzed using ATPase assays and examining the ability of the motor protein to drive the motion of actin filaments *in vitro*. To investigate the cellular functions of this phosphorylation, the authors reasoned that it is necessary to achieve better cellular stability of pSer19, so as to minimize the potential complexity that may result from active phosphatases in the cell. To this end, they synthesized another ‘caged’ mRLC, in which the phosphoserine was replaced by thiophosphoserine, which can function like the phosphorylated species but is stable in the presence of phosphatases. By injecting the caged thiophosphorylated mRLC/myosin II into COS7 cells, and then using UV irradiation at levels that are not generally toxic, the authors showed that they can achieve spatial control over the release of the active myosin inside a living cell.

This study provides a chemical tool for the precise spatiotemporal control over the intracellular activity of myosin II. The self-organized assembly of cytoskeletal structures depends on regulatory cues that can be mechanical (e.g. a force that unbinds a protein from the cytoskeletal filament) or chemical (e.g. phosphorylation). The ability to activate myosin II using light is likely to play an important role in determining the function of active myosin at a particular cellular site, while by-passing the inputs from kinases and phosphatases. A key next step will be to determine to what extent the diffusion of light-triggered myosin II could compromise the spatial precision in using this approach. We are optimistic that application of this approach will shed new light on the function of myosin in dynamic cellular processes, such as cell migration and cytokinesis. It is also likely that this strategy will be extended to other motor proteins (e.g. dynein) whose complex regulation based on phosphorylation is likely to be important.

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Figure 1.

Light-mediated activation of caged myosin II. NPE-caged thiophosphoserine is incorporated in to the mRLC to replace Ser19. The caging group blocks myosin activation by mRLC. UV-irradiation at 365 nm releases the thiophosphate group and activates myosin. This active form of myosin cannot be 'turned off' by endogenous phosphatases.