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Strategies for the photo-control of endogenous protein activity

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Photo-controlled or 'optogenetic' effectors interfacing with endogenous protein machinery allow the roles of endogenous proteins to be probed. There are two main approaches being used to develop optogenetic effectors: (i) caging strategies using photo-controlled conformational changes, and (ii) protein relocalization strategies using photo-controlled protein–protein interactions. Numerous specific examples of these approaches have been reported and efforts to develop general methods for photo-control of endogenous proteins are a current focus. The development of improved screening and selection methods for photo-switchable proteins would advance the field.

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Introduction

Optogenetic tools allow complex, dynamic systems to be probed, and permit questions to be tackled that neither traditional genetic techniques, such as gene silencing and knockouts, nor biochemical techniques, such as diffusible enzyme activators or antagonists, have the spatial or temporal resolution to address. While some naturally occurring photo-switchable proteins may be used directly, most require some sort of engineering or design to serve as useful optogenetic tools. Protein engineers have successfully created new photo-switchable enzymes [1,2], photo-switchable DNA binding proteins [3,4], and switchable receptors [5]. By co-opting naturally photo-controlled protein–protein interactions, they have invented new ways of controlling transcription, recombination [6] and cell division [7].

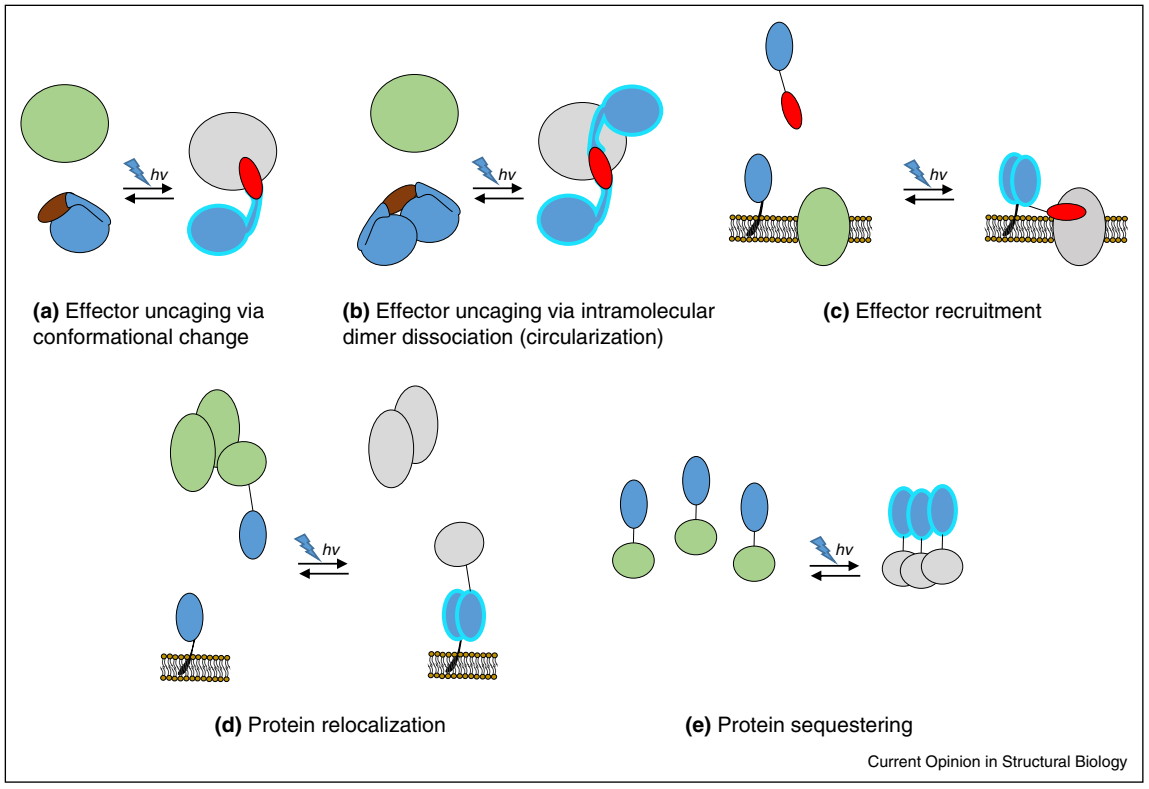
For this short review, we restrict our focus to optogenetic tools designed to interact with endogenous cellular proteins. Such optogenetic tools allow the role of a given protein to be probed in its native environment. They can be used to untangle the layers of regulation controlling protein activity in complex systems and offer exciting possibilities for basic research while at the same time clarifying the roles of potential therapeutic targets.

Photo-controlled effectors

A conceptually straightforward strategy for controlling the activity of an endogenous protein takes a known effector (usually an inhibitor, but sometimes an activator) and aims to control the availability of the effector by fusing it to a photo-switchable protein. The strategy is outlined in Figure 1a. The effector can be highly specific, interacting with a single endogenous target, or it can interact differentially with a set of targets. An advantage of this type of optogenetic tool is that it is a single component and requires no balancing of the expression of multiple components. In addition, there is no requirement to knockout an endogenous activity.

In such an approach, light-induced structural changes of a photo-switchable protein are used to control the target affinity of the effector by creating or alleviating steric hindrance — termed 'caging'. Photo-switchable proteins that exhibit large light-dependent conformational changes are employed, primarily the light-oxygen-voltage (LOV) domain of phototropin-2 from *Avena sativa* (and *Arabidopsis thaliana*) [8], but also photoactive yellow protein (PYP) from *Halorhodospira halophila* [9]. The AsLOV domain contains a flavin mononucleotide (FMN) chromophore that forms a thiol-adduct with a proximal cysteine when irradiated with blue light. A signal propagates through the protein core, triggering the undocking and partial unfolding of the C-terminal helix, termed the Jα-helix [8]. In the absence of blue light, the thiol-adduct decays and the Jα-helix re-folds and docks on the protein core. PYP absorbs blue light through a covalently bound *p*-coumaric acid-based chromophore, resulting in chromophore isomerization and a protein structural rearrangement involving the N-terminal cap [9]. PYP re-folds spontaneously in the dark. Recently, a circularly permuted PYP scaffold was developed that is expected to expand the potential for caging peptides using PYP [10]. An alternative strategy for caging was introduced by Lin and colleagues. Both ends of a target are fused to a pair of photo-switchable proteins based on Dronpa that dimerize, and thereby circularize the target [11•]. Irradiation leads to dimer dissociation and

Figure 1



Strategies for the photo-control of endogenous protein activity. **(a)** Inhibition of an endogenous protein through uncaging of an inhibitor. **(b)** Inhibition of an endogenous protein through uncaging of an inhibitor that had been inactivated via circularization. **(c)** Light-induced recruitment of a photo-active protein domain tagged to an inhibitor leads to inhibition of a local endogenous protein. **(d)** Inhibition of endogenous proteins through disruption of protein complexes by light-induced protein relocalization to an anchored photo-active protein domain. **(e)** Light-induced oligomerization of photo-active protein domains leads to inhibition of a tagged endogenous protein through sequestering. (Blue: photo-active domain, Green: active endogenous protein, Grey: inhibited endogenous protein, Red: inhibitor (Dark red: unavailable inhibitor), Black: membrane anchoring tag. A blue glow indicates light-activated state of the photo-active protein domain.).

enhanced availability of a binding interface. The concept is illustrated in Figure 1b.

Light-induced unfolding of the AsLOV Jα-helix was first used to control endogenous protein activity by Wu and colleagues [12]. The small GTPase Rac1 was fused to the Jα-helix in such a way that its target binding surface was sterically blocked in the dark-state, but exposed in the unfolded light-state. This construct, termed PA-Rac1, showed a ten-fold increase (2–0.2 μM) in affinity for the target PAK upon blue light irradiation. The change in Rac1 accessibility was sufficient to produce dramatic effects on cytoskeletal dynamics. When PA-Rac1 was activated by blue light at one edge of a mouse embryonic fibroblast cell, local protrusions were observed together with retraction on the opposite side of the cell. A point mutant of Rac1 (T17N) that is known to act as a dominant negative by binding and sequestering Rac guanine nucleotide exchange factors (GEFs) was caged by fusing it in the same manner to the Jα-helix of AsLOV. Irradiation of PA-Rac1-T17N led to localized retraction of the cytoskeleton and protrusion elsewhere in the cell. These constructs have now been used in a variety of settings to study Rac1 activity [13,14].

The concept of caging an effector using a photo-switchable protein has since been advanced by several groups. Kuhlman and colleagues [15] designed a chimeric fusion that embedded a peptide derived from the *Shigella* protein ipaA in the LOV Jα-helix. Upon light-induced unfolding of the Jα-helix, this peptide binds to the cytoskeletal protein vinculin and may be useful as a tool for studying the role of vinculin dynamics in cell motility. The same group developed fusions between the SsrA peptide and the LOV Jα-helix [15,16•]. The SsrA peptide binds the protease delivery protein SspB in *E. coli*, and can be used for photo-controlled protein relocalization in mammalian cells (*vide infra*). In developing these AsLOV-based photo-controlled effectors, the Kuhlman group made extensive use of computational protein design methods, as well as phage display of focussed AsLOV libraries to develop photo-controlled effectors with enhanced dynamic range (~50-fold changes in K_d) [16•].