Designing Chimeric LOV Photoswitches

G. Andrew Woolley[1,*]
[1]Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON M5S 3H6, Canada
*Correspondence: awoolley@chem.utoronto.ca
DOI 10.1016/j.chembiol.2012.04.003

The LOV domain from *Avena sativa* has a C-terminal (Jα) helix that dissociates and unfolds when the protein is exposed to blue light. Using computational protein design methods, Lungu et al., in this issue of *Chemistry & Biology*, created chimeric Jα sequences that show photo-controlled interactions with chosen targets.

Optogenetics, the application of genetically encoded light-switchable proteins to manipulate biological systems, is proving to be a transformative tool in fields where questions about the precise spatial and temporal regulation of biomolecular activity are central (Fenno et al., 2011). Whereas light sensitive ion channels are the photoswitches of choice for controlling excitable systems such as neurons, there are numerous cases where a non-membrane-bound photoswitch is required. For these sorts of targets, the LOV domain is quickly becoming a workhorse.

The LOV domain is a small water soluble protein that binds the cofactor flavin mononucleotide (FMN) (Figure 1) (Losi and Gärtner, 2011; Möglich and Moffat, 2010). Like the retinal chromophore used by channelrhodopsins, FMN is naturally present in cells and does not need to be added exogenously. Absorption of a blue photon by a LOV domain triggers a photoreaction in which a Cys residue reacts with the FMN, leading to protein conformational changes. The most conspicuous change is the dissociation and unfolding of a C-terminal helix (the *Jα* helix; Figure 1). Left in the dark, the cysteinyl adduct spontaneously reverts to FMN and Cys, and the *Jα* helix repacks against the protein core. Thus, the LOV domain represents a well-behaved, water-soluble, reversible, genetically-encoded photoswitch.

A key challenge for protein engineers is to effectively couple this photoswitch to a target protein of interest in order to regulate the activity of the target protein. Several ingenious schemes for doing this have been described including steric blockade, allosteric coupling, and mutual exclusive folding (reviewed by Möglich and Moffat, 2010). One fairly general strategy for effective coupling is to use photo-controllable interactions between the LOV domain and a naturally-occurring partner protein to either initiate or disrupt other protein-protein interactions (Wang et al., 2012; Yazawa et al., 2009). In the powerful TULIP (tunable, light-controlled interacting protein tags for cell biology) strategy just reported by Strickland et al. (2012), a PDZ domain binding peptide was connected to the LOV *Jα* helix to achieve versatile photo-control of protein localization. To manipulate certain types of targets, however, and to probe detailed mechanisms, one would like to directly manipulate the activity of naturally resident proteins such as transcription factors controlling developmental processes or proteins involved in actin remodeling underlying cell movement. For such cases, effective coupling of the photoswitch to the target protein becomes a specific problem in protein engineering.

Lungu et al. (2012), in this issue of *Chemistry & Biology*, bring some of the tools of the protein engineer, specifically structure-based design using the Rosetta computational modeling suite (Kaufmann et al., 2010) to the control of vinculin, a protein involved in forming the adhesions between the actin cytoskeleton and the extracellular matrix that are important for cell spreading and movement (Carisey and Ballestrem, 2011). Lungu et al. (2012) chose the LOV2 domain of *Avena sativa* phototropin 1 as a photoswitch, which has the advantage that biophysical analyses have characterized the kinetics and thermodynamics of the C-terminal *Jα* helix conformational change in great detail (Yao et al., 2008).

Their strategy was to make a chimeric version of the *Jα* helix that retained critical residues for photoswitch function but that also could function as a vinculin binding sequence when released from the LOV core upon irradiation. The problem is essentially a two-state protein design challenge in which one seeks to design a sequence that can alternate between two distinct conformational states with distinct binding partners. Using a Rosetta based approach, a LOV *Jα* /vinculin binding (ipaA) hybrid sequence was constructed as shown in Figure 1. The strategy proved very successful; with relatively little optimization, a chimeric *Jα* sequence was found that dissociates from the LOV domain upon irradiation and binds to the vinculin D1 domain. The enhanced affinity was almost exclusively determined by an enhanced on-rate. Greater availability of the chimeric helix in the light resulted in an approximately 50-fold increase in affinity for the vinculin D1 domain (from 245 nM [dark] to 5 nM [irradiated]).

While it remains to be seen if the resultant chimera is actually a useful tool for those interested in the spatiotemporal dynamics of cell spreading and movement, the protein design strategy employed is quite general. Indeed, the authors state that a search of the protein data bank for structures of peptides bound to target proteins reveals numerous sequences that have sufficient similarity to the LOV *Jα* helix that functional chimeras could be designed. Indeed they demonstrated the generality of the strategy by designing a LOV-SsrA peptide chimera. SsrA is a peptide sequence that interacts with the protease delivery protein SspB in *Escherichia coli*, and the LOV-SsrA chimera achieves an 8-fold photo-control of this interaction.

Thus, it appears that structure-based design of photoswitchable chimeras can be a viable general option for effective coupling. Of course there will be targets of interest where the particular primary sequence is simply unsuitable for making

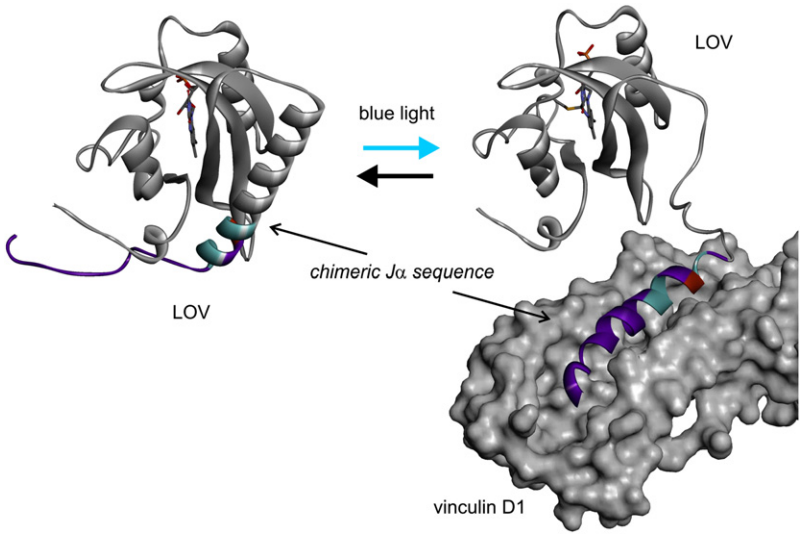

Figure 1. A Chimera of AsLOV2 and ipaA Shows Photo-Controlled Binding to Vinculin
The second LOV domain from *Avena sativa* (AsLOV2; gray ribbons) undergoes a light-triggered conformational change in which the flavin mononucleotide chromophore (sticks) reacts with a Cys residue and the *Jα* helix dissociates and unfolds (models are based on Protein Data Bank files 2V1A, 2V1B, and 2GWW). A chimeric *Jα* sequence can be constructed that binds to the LOV core in the dark and a chosen target protein upon irradiation. Lungu et al. (2012) show that a chimera of the *Jα* helix and the vinculin binding sequence ipaA can be designed when 50-fold changes in affinity for the target protein (gray surface) result upon irradiation. The chimeric *Jα* sequence is color coded as follows: blue, residues that are identical in both the AsLOV2 *Jα* and the ipaA; purple, residues from the ipaA domain; orange, residues from neither sequence designed to be compatible with both.

a chimera with the LOV *Jα* helix. In such cases, it may be that LOV domains from different sources could be employed. Alternatively, entirely different photoswitchable scaffolds are available. Morgan et al. (2010) have shown that the chimeras of photoactive yellow protein and the coiled-coil domain from GCN4 can be effective photoswitches. In general, a very broad range of potential targets could be subject to genetically-encoded photo-control if other genetically encoded switch domains that have different sorts of conformational changes and or sequence requirements can be characterized at the level of detail that has been achieved for the *A. sativa* LOV2 domain.

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