Hold me tightly LOV

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Mutations that increase the dynamic range of a photoswitchable protein may be used to improve other such photoswitches and increase their potential for allosteric control of protein activity in live cells.

The light-oxygen-voltage (LOV) protein domain has been recently used to control protein activity in living cells with light. In this issue of *Nature Methods*, Strickland *et al.* engineer important changes to improve the photophysical properties of this domain[1].

The picture of cell signaling as a fixed set of interlinked pathways has given way to a dynamic subcellular world in which the transient localization and kinetics of protein behavior determine the connections of signaling pathways. Activating the same protein in different locations, or with subtly different kinetics, can lead to diametrically opposed cell phenotypes (for instance, death versus proliferation). This shift in our view of cellular signaling has largely been driven by the ability to generate and visualize fluorescent proteins that are genetically encoded, such as GFP. This widened the study of protein dynamics from the specialized realm of laboratories in which researchers make dye-labeled proteins and inject them into living systems to the establishment of live-cell fluorescence imaging as a major component of modern cell biology.

Our ability to control proteins with the same level of spatial and temporal resolution in living cells has lagged far behind. The tantalizing view provided by modern imaging tools has spurred new research into protein 'caging'—releasing protein activity locally and with precise kinetics by irradiating a defined region of the cell. Small molecules and ions have been caged by derivatizing molecules or metal chelators with photocleavable moieties. Applying this approach to proteins is more difficult as they must be isolated, site-specifically labeled and reintroduced into cells without destroying their folding. Photocleavable protecting groups are cleaved irreversibly, and uncaging requires 'toxic' high-energy wavelengths capable of breaking covalent bonds. Most importantly, the approach does not offer the broad applicability and simplicity of genetically encoded proteins.

Recent years have seen the rapid growth of a new field, photoactivation of genetically encoded proteins in cells and animals. We are at an exciting stage, with introduction of multiple new strategies based either on derivatization of proteins with genetically encoded photocleavable moieties[2] or on creative engineering of naturally occurring light-responsive proteins. The Strickland *et al.*[1] paper in this issue describes improvements to a versatile light-responsive domain—the LOV2 domain from *Avena sativa* phototropin 1—that has been the basis for several such light-activated constructs (**Fig. 1**). In addition to the LOV domain, other approaches based on light-responsive proteins include light-regulated ion channels used to control nerve conductance[3] and light-mediated heterodimerization of proteins containing the Phy domain of *Arabidopsis thaliana*[4].

The α helix at the C terminus of the LOV domain (Jα helix) is coiled in the dark but becomes unwound upon irradiation and excitation of a flavin within the LOV domain. This large conformational change enables allosteric regulation of proteins that can be fused to the LOV domain. In one application, the LOV domain was attached to the N terminus of the GTPases Cdc42 and Rac[5]. Upon irradiation with blue light (450–500 nanometers), the Jα helix unwound, relieving steric inhibition by extending the 'tether' holding the LOV domain against the target

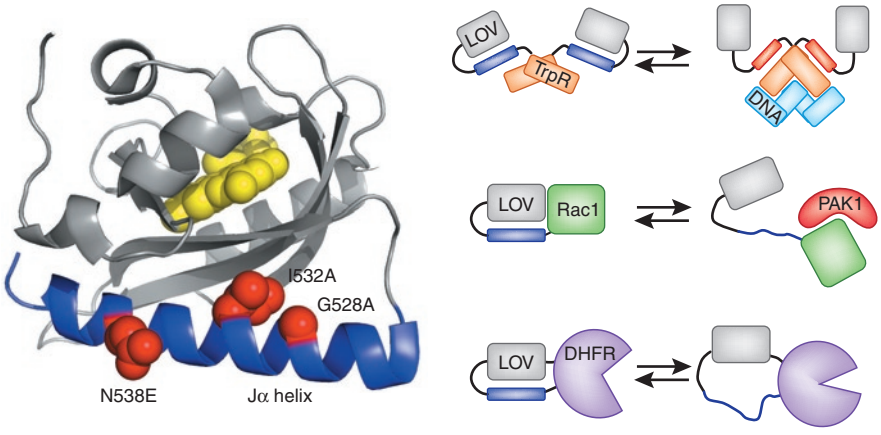

Figure 1 | LOV domain-based photoswitches. LOV domain structure (left). After activation of the flavin cofactor (yellow) with light, the Jα helix of the LOV domain (blue) undocks. Mutations identified by Strickland *et al.*[1] (red) stabilize the docked state and increase the dynamic range of a LOV domain–based photoswitch. Three LOV domain–based strategies have been previously used to control protein activity (right). Using LOV domain fusions, DNA binding of TrpR, PAK1 binding of the small GTPase Rac1 and activity of dihydrofolate reductase (DHFR) are controlled by light.

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protein. Caging was reversible and repeatable, and enabled control of cell movement in living animals[6]. The LOV domain has also been used to allosterically regulate the activity of the enzyme dihydrofolate reductase[7] and has been previously used by Sosnick and colleagues to cage a DNA-binding protein, the *Eschericha coli* trp repressor (TrpR)[8]. This design, called LovTAP, is an end-to-end fusion between the LOV domain and TrpR, and can only bind DNA when the Jα helix is undocked from the LOV domain.

The LOV domain–based photoswitches that have so far been constructed have exhibited modest changes in activity upon irradiation, between twofold and tenfold. However, experiments with the isolated LOV domain indicate that the open state (undocked Jα helix) is favored over the closed state (docked Jα helix) by an additional 3 kilocalories per mole when the LOV domain is irradiated, suggesting that it should be possible to create switches with larger changes in activity[9].

Strickland *et al.*[1] now postulate that first-generation LovTAP had only a fivefold change in activity because, in the dark, a fraction of LovTAP molecules still have an undocked Jα helix. To increase the light-dependent difference in the docked/undocked equilibrium, they identified mutations that stabilize a folded and docked Jα helix. The fraction of molecules with an undocked helix in the dark was reduced from 6% to below 1% for the isolated LOV domain. When they introduced these mutations into LovTAP, the affinity of the photoswitch for DNA was reduced tenfold in the dark compared to the first-generation LovTAP design, but the affinity for DNA in the light was similar. Overall, the 'dynamic range' of the switch was thus increased from 5- to 70-fold.

Because the identified mutations are located within the LOV domain and not the effector domain (TrpR), it is likely they will be useful for reducing dark-state activity in other engineered LOV domain–based switches that rely on undocking of the Jα helix. Indeed, residual dark activity has been apparent at high expression levels for current LOV-domain analogs. Additionally, these results suggest that the switching mechanism in the LOV domain is not oversensitive to mutations, so that it may be possible to find other mutations that act differently in the lit and dark states. Previous work has shown that point mutations in the flavin binding site can be used to control the kinetics of dark-state recovery by LOV domains, varying the lifetime for the open state from a few seconds to hours[10]. In future work, it will be exciting to see whether mutations can be rationally combined to create LOV domain–based switches that are tuned to have the appropriate dynamic range and kinetics for target applications.

Current engineered proteins based on different photoresponsive entities have complementary properties stemming from the photophysics of the light-responsive moiety. The Phy protein has a very fast uncaging and recaging rate compared to the LOV domain. It uses different wavelengths for uncaging and recaging, enabling very precise control, but two wavelengths require more complex instrumentation. The LOV domain is well suited for animal studies because only one wavelength is required and because of its naturally occurring flavin cofactor. Many imaginative and broadly applicable designs await us; these domains are capable of multiple forms of dimerization or conformational change.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Strickland, D. *et al. Nat. Methods* **7**, xxx–yyy **[Sosnick]** (2010).
2. Liu, C.C. & Schultz, P.G. *Annu. Rev. Biochem.* **79**, 413– **[AU: provide last page number]** (2010).
3. Airan, R.D., Thompson, K.R., Fenno, L.E., Bernstein, H. & Deisseroth, K. *Nature* **458** (7241), 1025– **[AU: provide last page number]** (2009).
4. Levskaya, A., Weiner, O.D., Lim, W.A. & Voigt, C.A. *Nature* **461**, 997– **[AU: provide last page number]** (2009).
5. Wu, Y.I. *et al. Nature* **461**, 104– **[AU: provide last page number]** (2009).
6. Yoo, S.K. *et al. Dev. Cell* **18**, 226– **[AU: provide last page number]** (2010).
7. Lee, J. *et al. Science* **322**, 438– **[AU: provide last page number]** (2008).
8. Strickland, D., Moffat, K. & Sosnick, T.R. *Proc. Natl. Acad. Sci. USA* **105**, 10709– **[AU: provide last page number]** (2008).
9. Yao, X., Rosen, M.K. & Gardner, K.H., *Nat. Chem. Biol.* **4**, 491– **[AU: provide last page number]** (2008).
10. Christie, J.M. *et al. Biochemistry* **46**, 9310– **[AU: provide last page number]** (2007).