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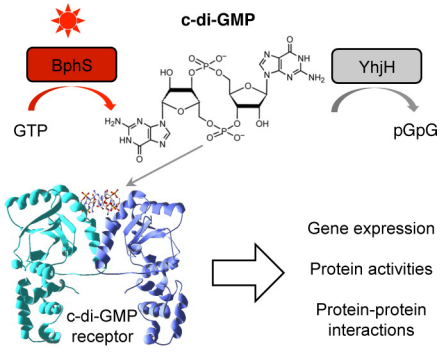
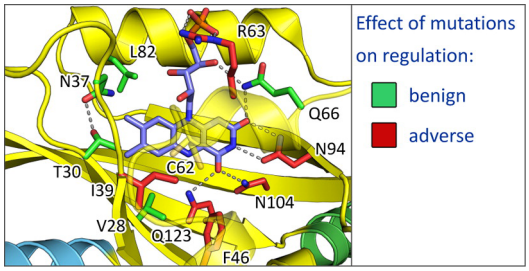
NEAR-INFRARED LIGHT RESPONSIVE SYNTHETIC c-di-GMP MODULE



Bacteriophytochromes sense light in the near-infrared region of the spectrum, where absorption by mammalian tissues is minimal. However, they can be controlled by external light sources in deep mammalian tissues, which makes them particularly attractive for cell-based therapeutics. Here, Ryu and Gomelsky (DOI: 10.1021/sb400182x) describe the design of a system where a bacteriophytochrome controls synthesis of a bacterial dinucleotide second messenger, c-di-GMP, which is not produced by animals, plants and certain microbes, and, therefore, amenable for orthogonal regulation.

Many protein and RNA sensors of c-di-GMP have been discovered and can be engineered as downstream modules to control gene expression, protein activities, and protein−protein interactions in a c-di-GMP-dependent manner. Thus, the synthetic c-di-GMP module presented here can be used for near-infrared light regulation of diverse biological activities in engineered mammalian or microbial cells in live animals.

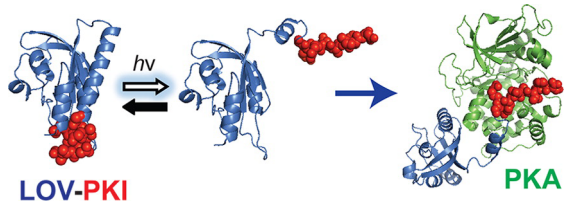
INVESTIGATION OF THE CHROMOPHORE-BINDING POCKET OF LIGHT-OXYGEN-VOLTAGE PHOTORECEPTORS



Light-oxygen-voltage (LOV) photoreceptors sense blue light and respond by changing their biological activity to elicit adequate physiological responses. As intrinsically light-switchable proteins, photoreceptors have widely been used in synthetic biology to precisely modulate cellular behavior and physiology. While several mutations in LOV proteins that modulate the time response and effective light sensitivity have been described, possible effects of these mutations on biological activity and regulation have not been investigated in detail. Now, Diensthuber and Engelhard et al. (DOI: 10.1021/sb400205x) report that many of these mutations severely impair activity and regulation whereas other mutations have no adverse effects.

The authors find that certain benign mutations affect the packing of hydrophobic residues in the interior of the LOV protein, thus presumably facilitating solvent access to the protein interior. Thus, mutations represent a powerful means for fine-tuning the time response and sensitivity of LOV photoreceptors but must be carefully chosen to avoid impairment of activity and regulation.

MANIPULATION OF ENDOGENOUS KINASE ACTIVITY IN LIVING CELLS



An enduring challenge for the field of cell signaling is the ability to distinguish between competing pathways in a cell. Phosphorylation sites within a substrate protein are often targeted by multiple kinases, and current methods lack the necessary precision to dissect the biological significance of competing pathways, especially in situations where spatiotemporal control of signaling is involved. Now, Yi *et al.* (DOI: 10.1021/sb5001356) have developed an approach with high spatiotemporal resolution that addresses these challenges.

The authors utilize light-dependent conformational changes in the LOV2 domain of *Avena sativa* phototropin 1 and demonstrate that inhibitory peptides for kinases can be controlled by light. This method is unique because it provides a minimally invasive method for perturbing endogenous signaling cascades in a subpopulation of cells, in addition to a method to inhibit kinase activity in specific subcellular locations.

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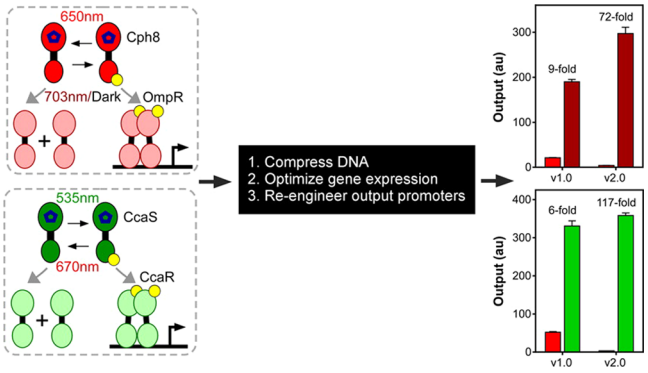
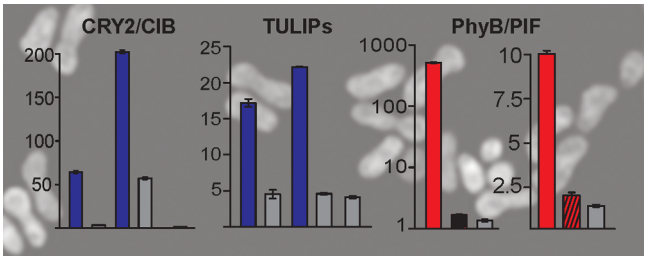
REFACTORING AND OPTIMIZATION OF LIGHT-SWITCHABLE BACTERIAL TWO-COMPONENT SYSTEMS



Bacterial two component systems (TCSs) are the most abundant sensing modality in nature, with over 75,000 identified in DNA sequence databases. However, most TCSs show relatively small gene expression responses to their input signals. Here, Schmidl *et al.* (DOI: 10.1021/sb500273n) demonstrate, for the first time, that all genes in a TCS pathway can be systematically optimized and that the output promoter sequences can be redesigned for lower leakiness and higher dynamic range.

The authors describe the systematic refactoring and optimization of previously described green/red and red/far red light-switchable bacterial two-component systems. The resulting sensors are each encoded in a highly compact manner on 2 plasmids, are compatible with the LacI, TetR and AraC systems, and have very low leakiness and greatly enhanced dynamic ranges of 72- and 117-fold. Thus, this study provides a new method for mining and optimizing virtually any TCS sensor from nature, which, in turn, can be used to engineer many other high performance sensors for synthetic biology.

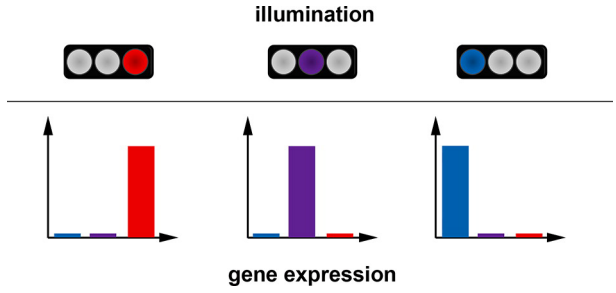
BENCHMARKING OF OPTICAL DIMERIZER SYSTEMS



Optical dimerizers are a powerful new class of optogenetic tools that allow light-inducible control of protein−protein interactions. In this study, Pathak *et al.* (DOI: 10.1021/sb500291r) focus on a systematic comparison of the properties of four different optical dimerizer systems, CRY2/CIB, TULIPs, phyB/PIF3, and phyB/PIF6, which were previously characterized by different groups. The authors compare the four systems in a yeast transcription assay and further compare the two blue light systems for their abilities to activate a yeast map kinase pathway. In the process, the authors also introduce an improved approach to use the CRY2/CIB system for local activation within cells and an improved method to control transcription in yeast with blue light.

This work allows for a better understanding of the capacities of these different dimerization systems and is of interest to the wide variety of users of different optical dimerizer systems.

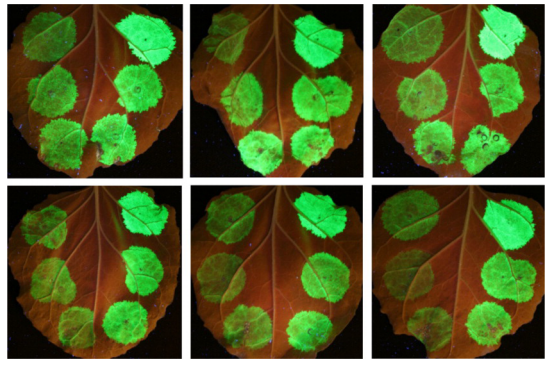
ORTHOGONAL OPTOGENETIC TRIPLE-GENE CONTROL IN MAMMALIAN CELLS



Over the last several years, the optogenetic toolbox for mammalian systems has been expanded rapidly and now includes several light-inducible gene switches that allow gene expression control with unprecedented resolution. However, due to significant overlaps of the absorbance spectra of photoreceptors, only a single report describing the use of multiple wavelengths to control the expression of three genes with partial orthogonality has been published. Here, Müller *et al.* (DOI: 10.1021/sb500305v) detail an approach that involves the mathematical model-guided development of a novel rapidly reversible blue light-inducible gene switch.

This approach yielded a setup that allowed the orthogonal control of three genes by illumination with UV-B, blue, and red/far-red light in a single mammalian cell culture. Since most biological processes are not controlled by the expression of a single gene but require the concerted action of multiple genes, the authors expect that their approach will enable the study of such processes with the spatiotemporal resolution of light. This multichromatic approach can also support the design of synthetic gene networks that can be controlled with high spatial and temporal resolution.

A GOLDEN GATE MODULAR CLONING TOOLBOX FOR PLANTS



Golden Gate cloning is a powerful and affordable modular system for DNA assembly. Like any modular system, it is much easier for laboratories to adopt if standardized, tested parts were easily available. To meet this need, Engler *et al.* (DOI: 10.1021/sb4001504) now provide a large toolbox of cloned, domesticated, and tested sequences.

The "Golden Gate Plant Toolkit" contains all the tools required for domestication of new sequences and assembly into single and multigene binary constructs. This kit contains domesticated standard parts including promoters, untranslated sequences, reporters, antigenic tags, localization signals, selectable markers and terminators.

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