

# UCSF

## UC San Francisco Previously Published Works

### Title

Spatiotemporal control of cell signalling using a light-switchable protein interaction

### Permalink

<https://escholarship.org/uc/item/2xh3m65p>

### Journal

Nature, 461(7266)

### ISSN

0028-0836

### Authors

Levskaya, Anselm  
Weiner, Orion D  
Lim, Wendell A  
et al.

### Publication Date

2009-10-01

### DOI

10.1038/nature08446

Peer reviewed



Published in final edited form as:

Nature. 2009 October 15; 461(7266): 997–1001. doi:10.1038/nature08446.

## Spatiotemporal Control of Cell Signalling Using A Light-Switchable Protein Interaction

Anselm Levskaya<sup>1,2,3</sup>, Orion D. Weiner<sup>1,4</sup>, Wendell A. Lim<sup>1,5,\*</sup>, and Christopher A. Voigt<sup>1,3</sup>

<sup>1</sup>The Cell Propulsion Lab, UCSF/UCB NIH Nanomedicine Development Center, University of California, San Francisco, California, 94158-2517

<sup>2</sup>Graduate Program in Biophysics, University of California, San Francisco, California, 94158-2517

<sup>3</sup>Dept. of Pharmaceutical Chemistry, University of California, San Francisco, California, 94158-2517

<sup>4</sup>Cardiovascular Research Institute, University of California, San Francisco, California, 94158-2517

<sup>5</sup>Howard Hughes Medical Institute and Dept. of Cellular and Molecular Pharmacology, University of California, San Francisco, California, 94158-2517

### Abstract

Genetically-encodable optical reporters, such as Green Fluorescent Protein, have revolutionized the observation and measurement of cellular states. However, the inverse challenge of using light to precisely control cellular behavior has only recently begun to be addressed; semi-synthetic chromophore-tethered receptors<sup>1</sup> and naturally-occurring channel rhodopsins have been used to directly perturb neuronal networks<sup>2,3</sup>. The difficulty of engineering light sensitive proteins remains a significant impediment to the optical control to most cell-biological processes. Here we demonstrate the use of a new genetically-encoded light-control system based on an optimized reversible protein-protein interaction from the phytochrome signaling network of *Arabidopsis thaliana*. Because protein-protein interactions are one of the most general currencies of cellular information, this system can in principal be generically used to control diverse functions. Here we show that this system can be used to precisely and reversibly translocate target proteins to the membrane with micrometer spatial resolution and second time resolution. We show that light-gated translocation of the upstream activators of rho-family GTPases, which control the actin cytoskeleton, can be used to precisely reshape and direct the cell morphology of mammalian cells. The light-gated protein-protein interaction that has been optimized in this work should be useful

---

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:[http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

\*Correspondence and request for materials should be addressed to W.A.L. (lim@cmp.ucsf.edu).

Supplementary Information is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

**Author Contributions** Concept was conceived by AL, WL and CV; experiments were executed by AL; spatiotemporal microscopy methods were developed by AL and OW; all authors were involved in interpretation of results and preparation of the manuscript.

Reprints and permissions information is available at [npg.nature.com/reprintsandpermissions](http://npg.nature.com/reprintsandpermissions).

The authors declare no competing financial interests.

for the design of diverse light-programmable reagents, potentially enabling a new generation of perturbative, quantitative experiments in cell biology.

---

A quantitative understanding of living cells will require methods to perturb and control the activities of their constituent proteins at fine spatial and temporal resolutions. By measuring responses to precise perturbations, predictive models of cellular networks can be tested and iteratively improved.<sup>4,5</sup> A promising approach is to couple the activity of targeted proteins to light signals, either by incorporating photoactive allosteric modulators semisynthetically<sup>1,6,7</sup>, or by exploiting naturally-occurring light-sensitive domains<sup>2,3,8-10</sup>. A particularly useful light-sensitive interaction for creating a general genetically-encoded light-control system for cell biology comes from the phytochrome signalling network of plants.

Phytochromes are photoreceptive signalling proteins responsible for mediating many light-sensitive processes in plants, including seed germination, seedling de-etiolation, and shade-avoidance.<sup>11</sup> They detect red and near-infrared light through the photoisomerization of a covalently-bound tetrapyrrole chromophore such as phycocyanobilin (PCB).<sup>11</sup> This photoisomerization event is coupled to an allosteric transition in the phytochrome between two conformational states called Pr (Red-absorbing) and Pfr (Far-Red-absorbing). (Fig. 1a) In one well-studied signalling pathway, upon stimulation with red (650 nm) light, the *Arabidopsis thaliana* phytochrome B (PhyB) protein binds directly to a downstream transcription factor, Phytochrome Interaction Factor 3 (PIF3), translocates to the nucleus as a heterodimer and directly modulates the transcription of response genes. PIF3 binds only the red-light exposed form of phytochrome, Pfr, and shows no measurable binding affinity for the dark- or infrared-exposed Pr state.<sup>12</sup> Thus, this interaction can be reversed by infrared light. This light-sensitive interaction has been mapped to the 650 residue N-terminal photosensory core of PhyB and a conserved 100 residue N-terminal Activated Phytochrome Binding (APB) domain of PIF3.<sup>13</sup>

In previous work, this light-sensitive interaction has been used in yeast to construct a photoreversible two-hybrid transcriptional activator to tune the expression level of a targeted reporter gene<sup>10</sup>, to target split intein domains to titrate the conditional protein splicing of a reporter gene<sup>14</sup>, and *in vitro* to directly target Cdc42 to its effector WASP to regulate actin nucleation.<sup>15</sup> Collectively this work suggests that the PhyB-PIF interaction can be functionally coupled to a wide variety of signalling processes through engineered fusion proteins.

To date, however, no reported system employing the PhyB-PIF interaction has been demonstrated to enable fine spatiotemporal control of dimerization *in vivo*. Indeed, the relatively weak binding strength and slow reverse kinetics of the reported domains<sup>15</sup> have prevented us from successfully applying these earlier interaction pairs for *in vivo* control of signalling. We have optimized the phytochrome interaction to enable its spatiotemporal control in experiments with live mammalian cells.

We first confirmed that PhyB could covalently bind externally supplied PCB chromophore in mammalian cells by utilizing a PhyB mutant (Y276H) that fluoresces at far-red

frequencies in the PCB-coupled state only.<sup>16</sup> NIH3T3 cells transfected with this construct show fluorescence after only 30min of exposure to 5 $\mu$ M PCB, confirming rapid autoligation at physiological conditions.(Fig. S1) Multiple potential phytochrome-PIF pairs were screened by a fluorescence translocation assay in NIH3T3 cells with confocal microscopy. We measured the red-light-induced translocation of Yellow Fluorescent Protein (YFP) fused to PIF domains to coexpressed phytochrome domains fused through a flexible linker to mCherry and localized to the plasma membrane by a C-terminal polybasic, prenylation sequence from Kras.<sup>17</sup> (Fig. 1b). Of all previously reported PIF domains<sup>13,18,19</sup>, only the N-terminus of PIF6 is strong enough to cause significant translocation of YFP to the membrane. (Fig. 1c) However, its interaction with the PhyB photosensory core (residues 1-650) is irreversible in infra-red light. Assaying it against different variants of PhyB revealed that the tandem C-terminal PAS domains of plant phytochromes are necessary to confer rapid photo-reversibility under infra-red light, underlining the importance of a previously reported autoinhibitory interaction for phytochrome signalling.<sup>20</sup> We refer to the optimized, reversible PhyB-PIF6 interaction simply as the “Phy-PIF” interaction.

Using this optimized Phy-PIF pair we observe rapid translocation to the plasma membrane under dilute red light (650nm 20 $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and from the membrane under infra-red light (>750nm 300 $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). (Fig. 2a, Supplemental Movies 1,2). Kinetic measurements of the Phy-induced cytoplasmic depletion of PIF-YFP under maximum illumination yield translocation time constants of 1.3 $\pm$ 0.1s (s.d. n=3) for membrane recruitment and 4 $\pm$ 1s (s.d. n=3) for membrane release (Fig. 2a, S2), demonstrating second-timescale control. These rates are an order of magnitude faster than previous chemically-induced translocation systems<sup>21</sup> and are very near the physical limits for whole-cell diffusion. (See Supplemental Calculation) The Phy-PIF translocation proved very robust—it could be cycled over a hundred times by alternating red and infrared illumination with no measurable decrease in recruitment ratios over time, despite many cycles of imaging at photon fluxes far higher than those phytochromes are exposed to in natural lighting conditions. (Fig. 2b, Supplemental Movie 3).

The rapid forward and reverse kinetics of our Phy-PIF pair allow for fine spatial control of membrane recruitment by simultaneously exposing cells to patterned light at the two antagonizing wavelengths. In NIH3T3 cells coexpressing the above Phy-KrasCAAX PIF-YFP recruitment pair, a nitrogen dye cell laser was used to deliver pulses of “activating” red light (650nm, 20Hz) to a focused point on the sample plane, while the whole sample was bathed in continuous “inactivating” infrared light obtained by filtering the microscope brightfield source (>750nm). (Fig. 3a) When the cell membrane is imaged by total internal reflectance (TIRF) microscopy we observe a sharp spot of membrane-localized YFP several microns in diameter around the irradiated point. (Fig. 3c) The rapid ‘off’ kinetics of the Phy-PIF interaction traps the membrane-recruited YFP pool to this spot, since any YFP diffusing away is dissociated from the membrane by the surrounding infrared light. This spot of recruited YFP can be rapidly relocated across the cell by repositioning the point of incident light. (Supplemental Movie 4)

We developed a second, fully automated method of controlling the distribution of both light frequencies on the cell membrane by using a digital micromirror array to project patterned

light onto the sample plane of the microscope at micron resolutions.<sup>22</sup> By irradiating the sample with 650nm and 750nm light sources oriented to take advantage of both micromirror angle states a complementary two-color red/infrared pattern can be projected onto the sample plane, allowing one to “paint” high-resolution inverse distributions of Pfr and Pr phytochrome onto the membrane of the cell. (Fig. 3b) We were able to faithfully project a simple pixel-based movie into the membrane-recruited PIF-YFP distribution of a NIH3T3 cell. TIRF imaging reveals fine features at five microns, demonstrating an unprecedented degree of control over protein localization in living cells.(Fig. 3d, Supplemental Movie 5). Additionally, by dithering the average amount of red-light in the target mask through software, we could smoothly titrate the fraction of active Phy and recruited PIF-YFP, demonstrating effective “greyscale” control of the chemical potential. (Fig. 3e, Supplemental Movie 6). Using this data, we estimate the in vivo dissociation constant of the PhyB-PIF6 interaction to be approximately  $K_d = 20\text{-}100\text{nM}$  (Fig. S5).

We were motivated to engineer a membrane recruitment system because many signalling proteins are, at least in part, activated by interactions that relocalize them to the membrane. Moreover, plasma membrane recruitment systems have been successfully used as a platform for small-molecule-induced chemical biology control systems.<sup>21,23,25</sup> For example, chemically induced membrane translocation of the rho- and ras-family small G-proteins<sup>21,23</sup> or the guanine nucleotide exchange factors (GEFs) that activate them<sup>21</sup> can generate global morphological changes. We reasoned that Phy-PIF induced translocation could generate similar morphological changes, but with much higher spatial and temporal resolution. We chose to focus on spatiotemporal control of the Rho-family GTPases Rac1, Cdc42, and RhoA given their central role in the dynamic spatial regulation of the actin cytoskeleton at the polarized edges of motile cells. (Fig. 4a)

Gated-recruitment constructs were made from the isolated catalytic modules (the DH-PH domain) of the Rac-GEF Tiam, the Cdc42 GEF Intersectin, and the Rho-GEF Tim. The optimal construct topologies for DHPH activation were found by screening for Tiam DHPH activity via the global morphological changes that occurred in transfected, serum-depleted NIH3T3 cells when the entire field was exposed to red light. Global recruitment of the optimal PIF-Tiam-DHPH chimera caused a pronounced lamellipodial phenotype within twenty minutes in the majority (>80%) of cotransfected cells, compared to PIF-YFP-only recruitment or control cells lacking the PCB chromophore. (Fig. 4b). This potent effect of recruiting the Tiam GEF activity to the membrane is similar to that observed using chemical dimerizers<sup>21</sup>. We further tested the generality of this construct topology by confirming that global RhoGEF recruitment induced cell body contraction in fibroblasts. (Supplemental Movie 9)

Given the strong global morphological effects of Tiam DH-PH domain membrane translocation, we then tested the effects of spatially localized light-activated translocation. Red laser stimulation was used for localized recruitment of the Tiam DH-PH domain in serum-depleted NIH3T3 cells (within a background of global repression by infrared light), effecting within 5-10 minutes a localized lamellipodial ‘bloom’.(Supplemental Movie 8) By slowly extending the point of activating light away from the cell, it is even possible to “draw out” an extended process up to 30 $\mu\text{m}$  from the main body of the cell that is stable after the

light has been withdrawn. This suggests the future possibility of programmatically specifying cell geometries and intercellular connections with light. (Fig. 4c, Supplemental Movies 7-8).

We further verified the signalling activity of our PIF-DHPH reagents by verifying that point induction causes local, transient increases of the active form of GTPase as measured by the membrane enrichment of biosensors—either mCherry tagged GBD binding domains from WASP (Fig 4d, Supplemental Movie 11) or PAK (Fig. S4)—by TIRF microscopy. Using these biosensors we see that GTPase activation occurs rapidly—within seconds—indicating that a subsequent signalling step is responsible for the typical delay of 5-10 minutes for lamellipodial and filopodial protrusions.

In summary, we have developed a genetically-encoded, light-switchable “Phy-PIF” interaction module which, because it has a properly titrated tight but reversible interaction, has the potential to be applied to control any live cell process that is dependent on a recruitment event. Unlike classical uncaging techniques, photoreversibility allows our system to defeat diffusive spreading by using patterned light. Further, the direct relationship between the recruited fluorescent fraction and signalling activity also enables measurable ‘dosage’ of signalling flux for quantitative perturbations. We show here that the system works robustly in mammalian cells with external PCB, extending previous demonstrations in yeast<sup>10</sup> and its natural domain in plants, suggesting that it is compatible with most eukaryotic cells. For genetically manipulable cells, it is in principle simple to include genes for enzymes that will generate PCB from heme or biliverdin.<sup>26</sup>

The high spatial and temporal resolution of light control allows this module to function as novel analytical tool, in which highly complex spatial or temporal patterns can be used to drive a process. We have also demonstrated here how this module can be used as a high resolution control module to sculpt cell shape in an unprecedented manner. Because of the generic nature of this interaction module, it is likely that it can be used to control an extremely broad range of cell biological processes without the need for laborious case-by-case protein engineering.

## Methods Summary

For detailed information on all methods, see Supplementary Information.

### Phycocyanobilin (PCB) Purification

PCB was extracted by methanolysis at 70°C from protein precipitates of *Spirulina* cell lysate (Seltzer Chemical) that were pre-washed to remove other tetrapyrroles species. Free PCB was handled under a green safelight ( $\lambda_{\max}$  550nm).

### Light Control Experiments

NIH3T3 cells transiently transfected with the phytochrome and PIF constructs were pre-incubated in the dark with 5 $\mu$ M PCB for 30min and then washed before experiments. Noncoherent control-light frequencies were obtained by filtering white-light sources with 650nm and 750nm 20nm bandpass filters (Edmund Optics) or a near-infrared RG9 glass

filter (Newport). For morphology experiments, cells were serum-depleted (1% Bovine Calf Serum) for at least twelve hours before imaging.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank Bassem El-Sady, James Tepperman, Kurt Thorn, Greg Kapp, and members of the Voigt, Weiner, and Lim laboratories of assistance and discussion. We thank Molecular Devices and Photonics Instruments for the loan and customization of a Mosaic spatial light modulator. Data for this study were acquired at the Nikon Imaging Center at UCSF. This work was supported by a NSFGR fellowship (A.L.); NIH R01 GM084040 and Searles Scholar Award.(O.D.W.); Packard Fellowship, the Howard Hughes Medical Institute, and NIH grants GM55040, GM62583, EY016546 (NIH Roadmap Nanomedicine Development Centers; [www.qb3.org/cpl](http://www.qb3.org/cpl)).(W.A.L.); Pew Fellowship, the Office of Naval Research, Packard Fellowship, NIH EY016546, NIH AI067699, NSF BES-0547637, UC-Discovery, and the SynBERC NSF ERC ([www.synberc.org](http://www.synberc.org)).(C.A.V.)

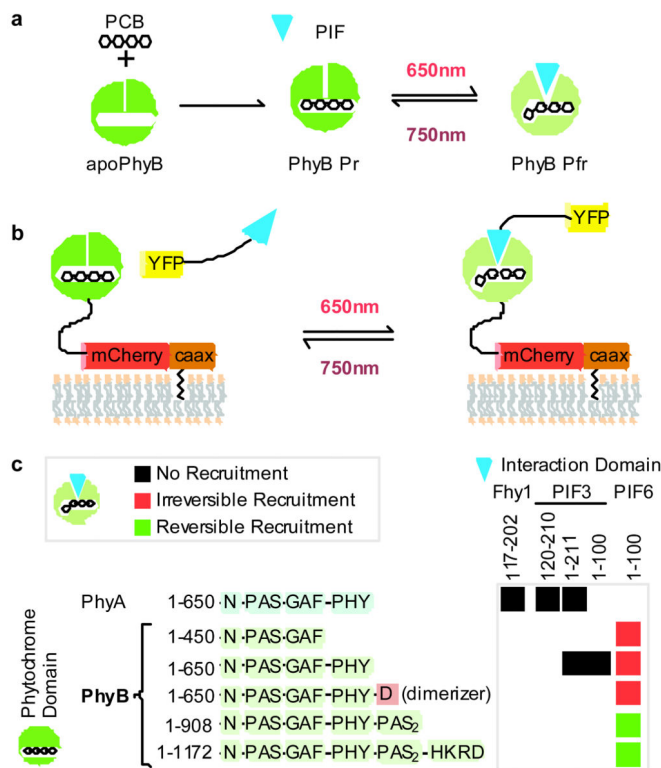
## References

1. Szobota S, et al. Remote control of neuronal activity with a light-gated glutamate receptor. *Neuron*. 2007; 54:535–45. [PubMed: 17521567]
2. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci*. 2005; 8:1263–8. [PubMed: 16116447]
3. Han X, Boyden ES. Multiple-color optical activation, silencing, and desynchronization of neural activity, with single-spike temporal resolution. *PLoS ONE*. 2007; 2:e299. [PubMed: 17375185]
4. Mettetal JT, Muzzey D, Gómez-Uribe C, van Oudenaarden A. The frequency dependence of osmo-adaptation in *Saccharomyces cerevisiae*. *Science*. 2008; 319:482–4. [PubMed: 18218902]
5. Bennett MR, et al. Metabolic gene regulation in a dynamically changing environment. *Nature*. 2008; 454:1119–22. [PubMed: 18668041]
6. Ghosh M, et al. Cofilin promotes actin polymerization and defines the direction of cell motility. *Science*. 2004; 304:743–6. [PubMed: 15118165]
7. Gorostiza P, Isacoff EY. Optical switches for remote and noninvasive control of cell signaling. *Science*. 2008; 322:395–9. [PubMed: 18927384]
8. Levskaya, et al. Synthetic biology: engineering *Escherichia coli* to see light. *Nature*. 2005; 438:441–2. [PubMed: 16306980]
9. Lee J, et al. Surface sites for engineering allosteric control in proteins. *Science*. 2008; 322:438–42. [PubMed: 18927392]
10. Shimizu-Sato S, Huq E, Tepperman JM, Quail PH. A light-switchable gene promoter system. *Nat Biotechnol*. 2002; 20:1041–4. [PubMed: 12219076]
11. Quail PH. Phytochrome photosensory signalling networks. *Nat Rev Mol Cell Biol*. 2002; 3:85–93. [PubMed: 11836510]
12. Ni M, Tepperman JM, Quail PH. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature*. 1999; 400:781–4. [PubMed: 10466729]
13. Khanna R, et al. A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. *Plant Cell*. 2004; 16:3033–44. [PubMed: 15486100]
14. Tyszkiewicz AB, Muir TW. Activation of protein splicing with light in yeast. *Nat Methods*. 2008; 5:303–5. [PubMed: 18272963]
15. Leung DW, Otomo C, Chory J, Rosen MK. Genetically encoded photoswitching of actin assembly through the Cdc42-WASP-Arp2/3 complex pathway. *Proc Natl Acad Sci U S A*. 2008; 105:12797–802. [PubMed: 18728185]

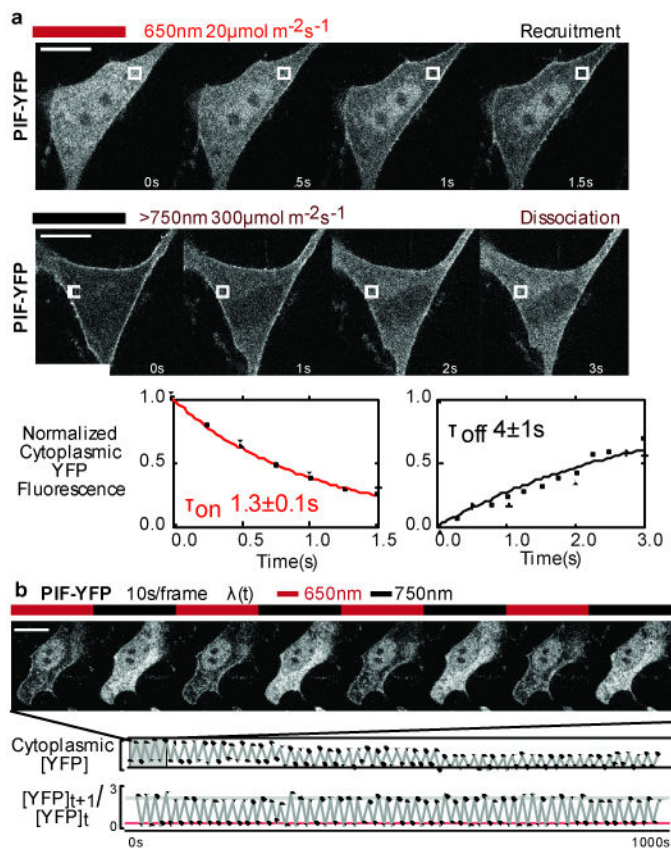


16. Su Y, Lagarias JC. Light-Independent Phytochrome Signaling Mediated by Dominant GAF Domain Tyrosine Mutants of Arabidopsis Phytochromes in Transgenic Plants. *Plant Cell*. 2007; 19:2124–39. [PubMed: 17660358]
17. Heo WD, et al. PI(3,4,5)P3 and PI(4,5)P2 lipids target proteins with polybasic clusters to the plasma membrane. *Science*. 2006; 314:1458–61. [PubMed: 17095657]
18. Al-Sady B, Ni W, Kircher S, Schäfer E, Quail PH. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. *Mol Cell*. 2006; 23:439–46. [PubMed: 16885032]
19. Genoud T, et al. FHY1 mediates nuclear import of the light-activated phytochrome A photoreceptor. *PLoS Genet*. 2008; 4:e1000143. [PubMed: 18670649]
20. Chen M, Tao Y, Lim J, Shaw A, Chory J. Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. *Curr Biol*. 2005; 15:637–42. [PubMed: 15823535]
21. Inoue T, Heo WD, Grimley JS, Wandless TJ, Meyer T. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. *Nat Methods*. 2005; 2:415–8. [PubMed: 15908919]
22. Wang S, et al. All optical interface for parallel, remote, and spatiotemporal control of neuronal activity. *Nano Lett*. 2007; 7:3859–63. [PubMed: 18034506]
23. Castellano F, et al. Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. *Curr Biol*. 1999; 9:351–60. [PubMed: 10209117]
24. Suh B, Inoue T, Meyer T, Hille B. Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. *Science*. 2006; 314:1454–7. [PubMed: 16990515]
25. Inoue T, Meyer T. Synthetic activation of endogenous PI3K and Rac identifies an AND-gate switch for cell polarization and migration. *PLoS ONE*. 2008; 3:e3068. [PubMed: 18728784]
26. Gambetta GA, Lagarias JC. Genetic engineering of phytochrome biosynthesis in bacteria. *Proc Natl Acad Sci U S A*. 2001; 98:10566–71. [PubMed: 11553807]

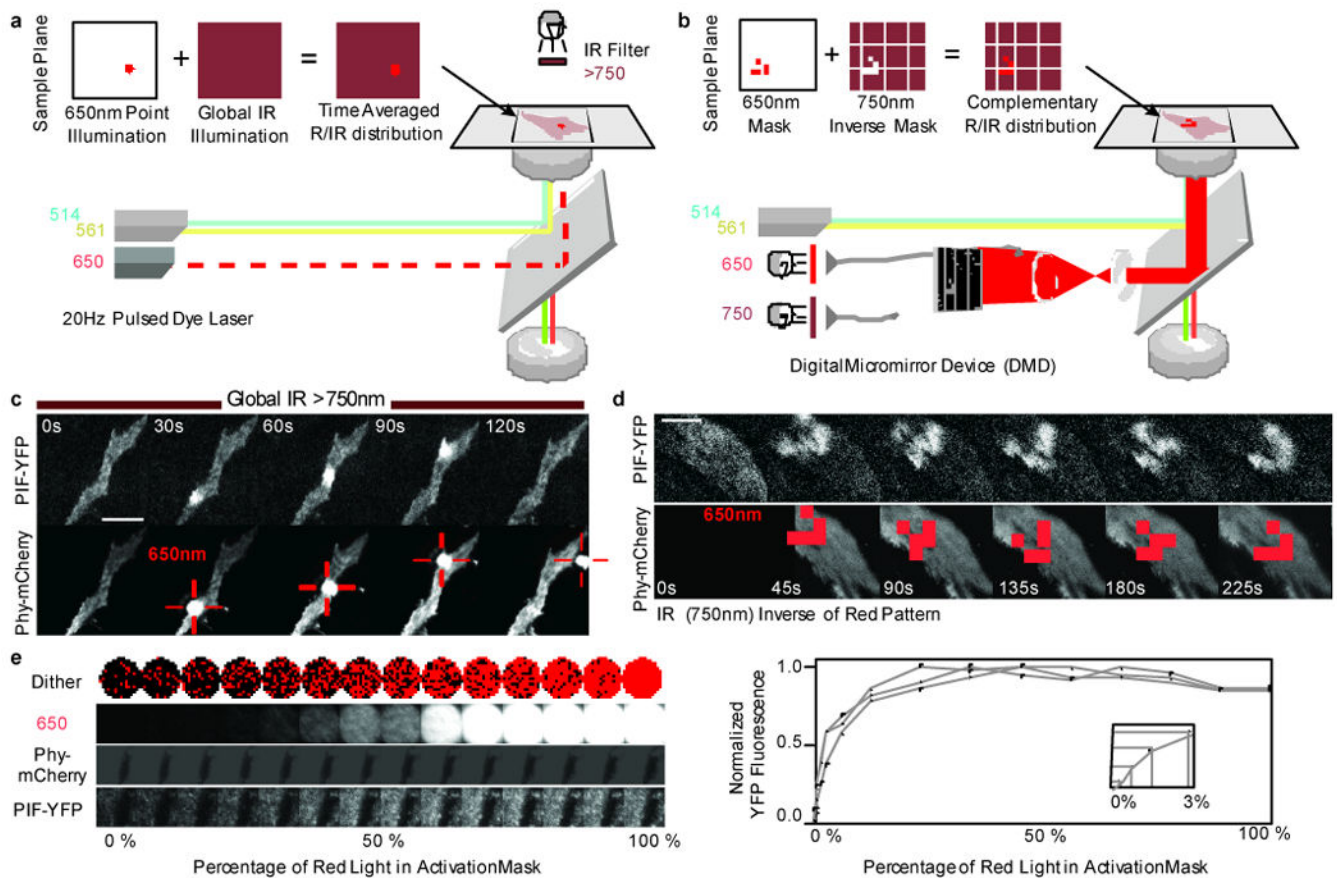




**Figure 1.** The phytochrome-PIF interaction can be used to reversibly translocate proteins to the plasma membrane in a light-controlled fashion. **a**, apoPhyB covalently binds to the chromophore phycocyanobilin (PCB) to form a light-sensitive holoprotein. PhyB undergoes conformational changes between the Pr and Pfr states catalyzed by red and infrared light, reversibly associating with the PIF domain only in the Pfr state. **b**, This heterodimerization interaction can be used to translocate a YFP-tagged PIF domain to PhyB tagged by mCherry and localized to the plasma membrane by the C-terminal caax motif of Kras. **c**, Phytochrome and PIF domains functional in mammalian cells were tested for reversible light-dependent recruitment of YFP to the plasma membrane using confocal microscopy. Previously published PIF constructs either failed to show visible recruitment or showed irreversible recruitment. Only PhyB constructs harboring tandem PAS repeats (unique to the plant phytochromes) showed detectable but reversible recruitment in vivo.

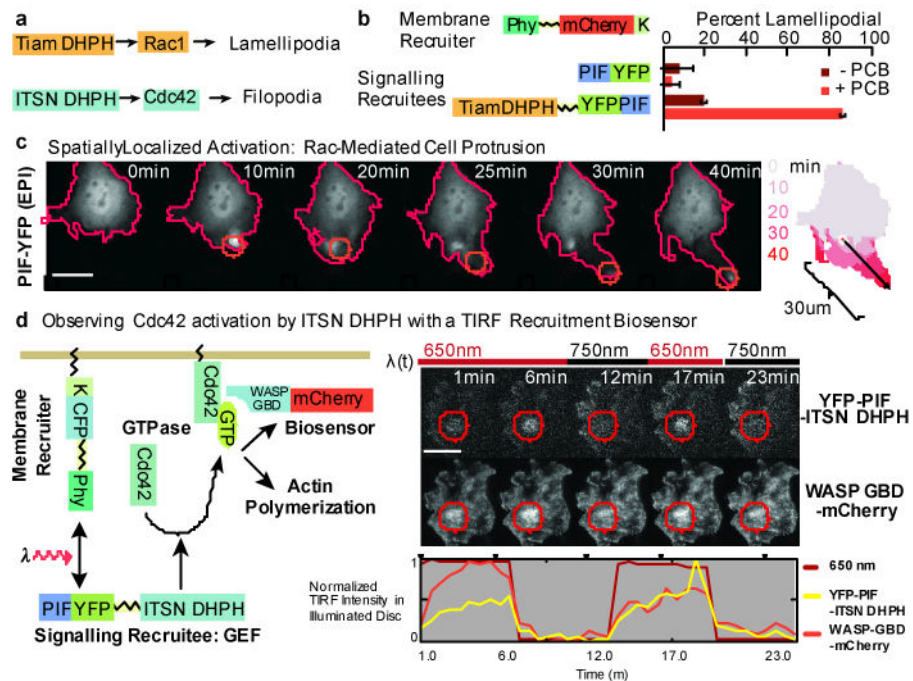


**Figure 2.** Confocal microscopy demonstrating the second-scale kinetics and photostability of the Phy-PIF photoswitchable membrane recruitment system. **a**, Confocal microscopy of NIH3T3 cells reveals rapid translocation of YFP between cytosol and plasma membrane under red and infrared light. Fitting exponentials to the cytoplasmic depletion of YFP gives typical time-constants of  $1.3 \pm 0.1s$  for recruitment and  $4 \pm 1s$  for dissociation ( $n=3$ ). White rectangles show regions sampled for plotted traces. Arrows in graphs mark the timepoints shown. (Supplemental Movies 1,2) **b**, Rapid alternation between the 650 and 750 nm light can generate oscillations in the cytoplasmic concentrations of YFP. Absolute cytoplasmic concentration of YFP for this series is plotted along with the ratio change between time-points to adjust for photobleaching and cell-drift. The red and grey bars represent the standard deviations of the recruited and released cytosolic fluorescence, demonstrating near-fixed recruitment ratios over more than a hundred iterations. (Supplemental Movie 3) Scale bars 20 μm.



**Figure 3.**

Recruitment to the plasma membrane can be controlled spatially by simultaneously irradiating cells with patterned red and infra-red light. **a**, A nitrogen dye cell laser exciting a 650nm rhodamine dye was focused onto the sample plane of the microscope at 20Hz while IR-filtered white light continuously bathed the entire sample. **b**, A digital micromirror device focused onto the sample plane was used to send high-resolution patterns of 650nm/750nm light from a DG-4 source into the microscope under software control. This results in complementary red and infra-red distributions on the sample plane. **c**, TIRF imaging of localized membrane recruitment by a point source as in **a** shows highly localized YFP recruitment. (Supplementary movie 4) The recruited YFP spot's diameter is roughly  $3\mu\text{m}$  and can be quickly moved by repositioning the laser. The final frame shows that the YFP spot is not merely bleed-through of the excitatory laser light, but genuine local fluorescent protein recruitment. **d**, TIRF movies of structured membrane recruitment by programmatically updating masks for red and infrared light by a digital micromirror device as in **b** were collected, revealing a faithful reproduction in the recruited YFP distribution of a movie of the cellular automaton 'game-of-life glider' that was projected (Supplementary movie 5). **e**, Images show the raw traces of titrated input 650nm light and recruited PIF-YFP. The plot at left shows the recruitment level as a function of 650nm ratio for three typical experiments. Inset shows the non-saturated regime. Scale bars  $20\mu\text{m}$ .



**Figure 4.**

Rho-family G-protein signalling can be controlled by the light-activated translocation system. **a**, The catalytic DH-PH domains of RhoGEFs Tiam and Intersectin activate their respective G-proteins Rac1 and Cdc42 which in turn act through effector proteins to modify the actin cytoskeleton. **b**, Recruitte constructs with Tiam DH-PH domains were assayed for their ability to induce lamellipodia in NIH3T3 by exposing serum-depleted cells transfected with the indicated constructs to red (650nm) light and counting the percentage of cells that produced lamellipodia within 20min under live microscopy. Error bars s.e.m., (n=2, avg. 30 cells; p-value=.0004 for Tiam) **c**, Local induction and ‘extrusion’ of lamellipodia in live NIH3T3 cells was demonstrated by globally irradiating the whole sample with an infrared (750nm) light source while focusing a red (650nm) laser onto a small portion of the cell as in **2a** and slowly extending this red-targeted region from the cell body. Superimposed outlines of the cell show directed extension 30µm along the line of light movement. (Supplemental Movie 7) **d**, Cdc42-GTP binding domain (WASP-GBD) linked to mCherry was used to measure the “response function” of Intersectin DHPH recruitment over several iterations in time and in space at equilibrium. (Supplemental Movie 11) Scale bars 20µm.