Engineering an E. coli Near-Infrared Light Sensor

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Supporting Information

ABSTRACT: Optogenetics is a technology wherein researchers combine light and genetically engineered photoreceptors to control biological processes with unrivaled precision. Near-infrared (NIR) wavelengths (>700 nm) are desirable optogenetic inputs due to their low phototoxicity and spectral isolation from most photoproteins. The bacteriophytochrome photoreceptor 1 (BphP1), found in several purple photosynthetic bacteria, senses NIR light and activates transcription of photosystem promoters by binding to and inhibiting the transcriptional repressor PpsR2. Here, we examine the response of a library of output promoters to increasing levels of Rhodopseudomonas palustris PpsR2 expression, and we identify that of Bradyrhizobium sp. BTAi1 crtE as the most strongly repressed in Escherichia coli. Next, we optimize Rps. palustris bphP1 and ppsR2 expression in a strain engineered to produce the required chromophore biliverdin IXα in order to demonstrate NIR-activated transcription. Unlike a previously engineered bacterial NIR photoreceptor, our system does not require production of a second messenger, and it exhibits rapid response dynamics. It is also the most red-shifted bacterial optogenetic tool yet reported by approximately 50 nm. Accordingly, our BphP1–PpsR2 system has numerous applications in bacterial optogenetics.

KEYWORDS: synthetic biology, optogenetics, near-infrared light, bacteriophytochrome

In previous work, we and others have engineered bacterial photoreceptors that regulate transcription in response to ultraviolet (maximal wavelength (λmax) = 382–405 nm), blue (λmax = 450 nm), green (λmax = 535 nm), red (λmax = 650 nm), and short wavelength near-infrared (NIR; λmax = 712 nm) light. Several of these photoreceptors have been used to spatially pattern gene expression across macroscopic and microscopic cell populations, engineer a bacterial edge detector, induce permanent genetic memory via recombination expression, program tailor-made expression dynamics of one and two independent proteins, place protein expression under in silico feedback control, and reveal novel input/output dynamics of a widely used synthetic genetic circuit. Bacteriophytochromes (BphPs) are the most red-shifted biological photoreceptors known. BphPs contain an N-terminal photosensory core domain (PCD) and C-terminal effector domain that regulates the biological response. The PCD is composed of a Per/Arnt/Sim (PAS), cGMP phosphodiesterase/adenyl cyclase/FhlA (GAF), phytochrome (PHY) motif with the chromophore biliverdin IXα (BV) covalently bound near the N-terminus. Canonical BphPs adopt an inactive ground (i.e., dark-adapted) conformation with maximal absorption between 690 and 710 nm. This ground state is referred to as P0 for phytochrome red absorbing state, by literature convention. Upon exposure to red or short wavelength NIR light, these BphPs switch to the biologically active P(α)(BV) covalently bound to produce BV. When activated by red or short wavelength NIR, the BphG1–Slr1143 chimera (named BphS) synthesizes cyclic di-GMP (c-di-GMP), a bacterial second messenger. MrkH, a Klebsiella pneumoniae transcription factor, binds this c-di-GMP, resulting in transcription from the PmrA output promoter with a reported 40-fold dynamic range (ratio of reporter gene levels in the activated versus inactive state).

Despite its utility, BphS–MrkH has several limitations. First, heterologous production of c-di-GMP has been shown to alter the expression of approximately 200 E. coli genes, including those involved in the central processes of sugar metabolism and cell-wall modification. c-di-GMP also controls biofilm

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Figure 1. Engineering PpsR2-repressible E. coli promoters. (a) Annotated sequences of 10 candidate promoters. (b) Device schematic for the PpsR2 induction and promoter activity measurement plasmid system. (c) Promoter transfer functions. Dashed lines indicate the maximal cellular fluorescence levels. Data points represent the total fluorescence levels of cells carrying the promoter–reporter plasmid and pNO15. Data are from experiments on three separate days. Error bars represent the standard deviation (SD) of the mean. Solid lines represent the deactivating form of the Hill function, \( f(I) = b - \frac{a}{I^{1/2}} \), fit to each data set, where \( I \) is the concentration of IPTG (mM), \( b \) and \( a \) are the fit parameters for the initial and range of sfGFP (MEFL) output values, respectively, \( K_{1/2} \) represents the concentration of IPTG (mM) needed to reach half-maximal repression of the promoters, and \( n \) is the Hill coefficient. Fit parameter values are listed in Table S2. E. coli cell autofluorescence is denoted by the gray area.
formation,26 cell division,27 the production of extracellular curli protein fibers,28 polynucleotide phosphorylase activity,29 and flagellar rotation bias.30 Second, bacterial genomes encode many poorly characterized c-di-GMP synthases and phosphodiesterases (e.g., *E. coli* encodes 12 and 13, respectively) that could cross-activate or cross-repress the $P_{mrkA}$ output promoter in unwanted or unknown ways.31 Third, BphS$^{-}$MrkH requires 1.5 h after exposure to activating light to produce an observable gene expression response and 6 h to reach half-maximal activation ($t_{1/2}$). Most model bacterial strains double in 0.3−1 h under laboratory conditions, and many regulatory processes occur on these time scales as well.32 Thus, these slow dynamics make BphS$^{-}$MrkH a poor choice for characterizing the dynamics of many pathways.33 Finally, BphS is likely to be spectrally incompatible with red light responsive photoreceptors as well as red-shifted fluorescent proteins. For example, Ryu and Gomelsky activated BphS$^{-}$MrkH with 660 nm light emitting diodes (LEDs),10 which strongly repress the widely used *E. coli* green light sensor CcaSR and red light sensor Cph8−OmpR ($\lambda_{\text{max}} = 650$ nm).15 Additionally, imaging of the commonly used infrared fluorescent protein IFP1.4 (excitation maximum = 684 nm)34 would likely cross-activate BphS$^{-}$MrkH.

*Rhodopseudomonas palustris* CGA009 BphP1 is a bathy BphP with a more red-shifted ground state absorption spectrum ($\lambda_{\text{max}} = 760$ nm) than that of any previously reported bacterial optogenetic tool.35 BphP1 possesses a C-terminal HOS (2-helix output sensor) effector domain that is inactive in the Pfr state, but it binds and inactivates the transcriptional repressor PpsR in the Pr conformation.35 PpsR binds to TGT-N12-ACA operator sites that often overlap the −35/−10 regions of $\sigma^{70}$-type promoters that drive the expression of genes involved in photosynthesis.36,37

To engineer BphP1−PpsR2 to function as an optogenetic tool, we first developed a strong *E. coli* promoter that is highly repressed by PpsR2 expression. Next, we optimized BphP1 and PpsR2 expression and BV production to achieve the desired light response. Finally, we performed kinetic and spectral experiments to demonstrate that our BphP1−PpsR2 system responds to changes in light input in minutes and exhibits the

Figure 2. Engineering BphP1−PpsR2 to function as an *E. coli* photoreceptor. (a) Device schematic for the chemically inducible BphP1−PpsR2 system. (b) Dependence of system response on BphP1 and PpsR2 expression. (c) Device schematic for the hardwired BphP1−PpsR2 system. (d) Response of hardwired system and negative controls to NIR, R, and dark. WT: wild-type. bphP1ΔHOS: deletion of BphP1 HOS domain. ppsR2ΔDBD: deletion of PpsR2 DNA binding domain. Δho1: deletion of BV production plasmid. Bar heights represent the mean of experiments performed on three separate days. Error bars represent the SD of the mean. Reported sfGFP values are corrected for cell auto-fluorescence. Statistically significant differences are annotated as such: *p < 0.05; **p < 0.01 (two-way ANOVA followed by Tukey’s multiple comparison test). (e) Representative flow cytometry histograms of the data in panel D.
most red-shifted action spectrum of any bacterial optogenetic tool.

### RESULTS AND DISCUSSION

**Engineering PpsR2-Repressible E. coli Promoters.** To engineer a BphP1–PpsR2 output promoter in *E. coli*, we considered the intergenic regions upstream of bchC, crtD, and crtE. We chose these promoters because they have been validated to be directly bound by PpsR2 in DNA footprinting experiments.38 We examined sequences from both *Rps. palustris* and the closely related *Bradyrhizobium* BTAi1, which has a homologous BphP1–PpsR2 pair.39 Using the BPROM webtool,40 we identified a single putative *E. coli* promoter with one PpsR2 operator overlapping the −35 site and a second overlapping the −10 site in all six intergenic regions (Figure S1). BTAi1 bchC contains a third operator upstream of the −35, and *Rps. palustris* crtD and crtE each possess one additional semiconserved operator (TGA-N12-ACA) near the transcriptional start site (+1). Each +1 site is separated from the start codon of the downstream ORF by 30–50 bp S′ untranslated regions (UTRs) (Figure S1).

Next, we redesigned each of these promoter regions to function reliably in *E. coli*. First, to reduce the possibility of unwanted regulation by alternative pathways, we truncated all DNA greater than 10 bp upstream of the −35 or most distal PpsR2 operator (Figure S1). Additionally, we deleted all DNA downstream of each terminal promoter element (i.e., +1, or third site operator) to eliminate any possible regulatory impact from the S′ UTRs. We named the resulting minimized promoters PBr_bchC, PBr_crtD, PBr_crtE, PBr_bchC, and PBr_crtE to indicate the organism and gene from which they are derived. Because the third operator sites of PBr_bchC, PBr_crtD, and PBr_crtE do not predictably overlap PpsR2 −35/−10 elements and may thus contribute less to transcriptional repression in our experimental system, we also designed versions of each wherein these operators are deleted (Pbr_bchC, Pbr_crtD2, and Pbr_crtE2) (Figure S1). Finally, we designed a synthetic PpsR2-repressible *E. coli* promoter by replacing the cl operator sites in the strong bacteriophage β promoter Pβ41 with PpsR2 operator sites. This promoter, which we named PpsR2, is based on the design of the strongly repressible engineered tetracycline repressor (TetR)-repressed promoter P_L_TetR42 (Figure S2).

To characterize the resulting library (Figure 1a), we next constructed a series of plasmids (pNO1–10) wherein we encoded superfolder green fluorescent protein (sfGFP)33 downstream of each candidate promoter along with a computationally designed synthetic *E. coli* ribosome binding site (rBS)43,44 (Figures 1b and S3). Then, we transformed each plasmid into *E. coli* and measured the resulting sfGFP expression levels by flow cytometry (Methods). Though Pbr_crtD and Pbr_crtE2 are very weak, the remaining eight promoters are clearly functional, driving total cellular fluorescence levels between 523 ± 17 and 6900 ± 230 (P_br_crtE) molecules of equivalent fluoroscein (MEFL) compared to the *E. coli* autofluorescence level of 165 ± 10 MEFL (Figure 1c). PBr_bchC2 and PBr_crtE2 are weaker than their parental promoters, demonstrating that the region containing the third operator enhances the overall transcription rate (Figure 1c).

Next, we characterized the extent to which each promoter is repressed by PpsR2 expression. To this end, we constructed plasmid pNO15, wherein *Rps. palustris* ppsR2 is transcribed under inducible control of isopropyl β-D-1-thiogalactopyranoside (IPTG) (Figure S3). Then, we cotransformed pNO15 pairwise with each member of the pNO1–10 set, grew the resulting strains in the presence of variable levels of IPTG, and measured sfGFP levels as before. As expected, transcription from each of the eight functional promoters decreases with PpsR2 induction (Figure 1c). P_br_crtE (encoded on pNO4) again exhibits the greatest response, with a 76 ± 20-fold dynamic range (Figure 1c). Accordingly, we selected P_br_crtE as the BphP1–PpsR2 output promoter for subsequent experiments.

**Engineering BphP1–PpsR2 To Function as an E. coli Photoreceptor.** The architecture of BphP1–PpsR2 is similar to that of bacterial two-component histidine kinase signal transduction systems (TCSs).46 We have previously shown that TCS performance is sensitive to the expression levels of both the sensor kinase (analogous to BphP1) and response regulator (analogous to PpsR2).18 To optimize BphP1–PpsR2 function in *E. coli*, we therefore added a cassette wherein *Rps. palustris* bphP1 is transcribed under control of an anhydrotetracycline (aTc)-inducible promoter to pNO15, resulting in pNO17 (Figures 2a and S4). To engineer *E. coli* to produce BV, we also engineered plasmid pNO41, from which a heme oxygenase gene is constitutively expressed (Figures 2a and S4). We optimized the expression levels of a heme oxygenase gene on pNO41 to maximize BV production (Figure S5).

To assay the function of this engineered system, we grew *E. coli* carrying pNO4, pNO17, and pNO41 in various concentrations of aTc and IPTG while exposing them to either 760 nm (NIR) or 660 nm (red) light (Methods). Indeed, we observed NIR activation under a wide range of BphP1 and PpsR2 expression levels, with a maximum activation of 2.35 ± 0.28-fold (Figure 2b).

While convenient for preliminary system validation, the requirement for IPTG and aTc limits the compatibility of optogenetic tools with many other engineered genetic systems that utilize these inducers or their target repressor proteins.5 Therefore, we next replaced the two inducible promoters with a constitutive version driving a synthetic bphP1–ppsR2 operon (Figures 2c and S4). To reoptimize BphP1 and PpsR2 expression levels, we designed eight sRBSs with variable predicted strengths for each gene (Table S1). We then constructed 64 plasmids (pNO131–194, Figure S4) to test the 8 × 8 matrix of sRBS strength combinations for these two genes (Table S1). Then, we cotransformed each plasmid with pNO4 and pNO41 and characterized the NIR light response as before. While more than half of the designs do not appreciably respond to light, approximately one-third exhibit between 1.1- and 2-fold NIR activation (Figure S6), and five yield a ≥2-fold response (Figure S6). The best performing system is encoded on pNO183 (Figure S4) and has a dynamic range of 2.47 ± 0.17-fold, similar to the best aTc and IPTG induction conditions.

To be certain that we had achieved the maximum dynamic range, we also tested the other seven PpsR2-repressible promoters with pNO183 and pNO41. However, these all yielded smaller dynamic ranges than P_br_crtE (Figure S7).

Next, we performed a series of control experiments to validate that the light response is due to BphP1–PpsR2 signaling rather than an alternative pathway. As expected, removal of the BphP1 HOS domain results in constitutively low sfGFP levels (Figure 2d). Similarly, deletion of the PpsR2 DBD results in constitutively high sfGFP (Figure 2d). Furthermore, the lack of BV abolishes the light response (Figure 2d). Dark treatment also results in intermediate sfGFP output relative to
NIR and R illumination (Figure 2d,e). These data are consistent with in vitro results demonstrating that dark-treated holo-BphP1 first adopts the P state and then gradually converts to P, which should generate an equilibrium between these two states in actively growing dark-treated cells. Taken together, these results clearly demonstrate that we have recapitulated the function of the BphP1–PpsR2 system in E. coli.

**Transfer Functions.** The ability to use variable light intensities or wavelength combinations to tune the output transcription rate enables researchers to program gene expression levels and dynamics with high quantitative precision, an important feature of optogenetic tools. Thus, we characterized the BphP1–PpsR2 transfer function, or steady-state response to increasing NIR intensity, in the presence of different intensities of deactivating red light. In the absence of red light, sfGFP output increases between 0 and 72 μmol/m²·s NIR light. The response is well-fit by a Hill function with an intensity resulting in 50% maximal response (K) equal to 8.63 ± 0.54 μmol/m²·s and a Hill coefficient (n) equal to 1.209 ± 0.091 (Figure 3). This Hill coefficient reveals that the system exhibits a relatively linear response over a wide range of light intensities, a desirable performance feature for tuning the transcription rate. As expected, the presence of red light reduces the NIR response (Figure 3).

By repeating the transfer function measurement in the presence of variable red light intensities (Figure S8), we observed that approximately 1.2 additional NIR photons are required to overcome the inhibitory effect of each red photon (Figure S9). This result suggests the system has similar sensitivity to NIR and red light, a feature that should make it easy to modulate transcriptional output under diverse experimental conditions.

**Input/Output Dynamics.** Next, we characterized the step–response dynamics of our engineered BphP1–PpsR2 system. Specifically, we preconditioned cells in saturating red light and then instantaneously switched to saturating NIR (step ON), performed the converse experiment (step OFF), and switched from saturating NIR to dark (step DARK). Then, we used our previously developed kinetic protocol based on the inhibition of transcription and maturation of sfGFP (Methods) to monitor system output over time (Figure 4).

We fit each data set to a simplified version of a previous ordinary differential equation model we developed to capture TCS photoreceptor response dynamics (Figure S10). This BphP1–PpsR2 model depicts a pure delay after a change in light input during which time the output promoter activity is constant (τ, min) followed by a change in promoter activity that obeys single exponential kinetics. For step ON, τ fits to 5.3 ± 0.3 min and the time required to reach half-maximal activation (t) to 26.9 (−5.4/+6.0) min (Figure 4; Methods). For step OFF, we estimate τ to be 8.6 ± 2.3 min and t as 38.2 (−4.3/+4.6) min (Figure 4). Finally, for step DARK, τ fits to 24.9 ± 2.9 min and t fits to 63.5 (−6.5/+7.4) min (Figure 4). These response times are similar to the time scales of bacterial gene expression and to the dynamics of our previous green, red, and UV sensors. The slower response for the step DARK is also consistent with in vitro measurements of a 50 min half-life for BphP1 dark reversion.

**Action Spectra.** The action spectrum(a) of an optogenetic tool depicts the relationship between input wavelength(s), intensity(ies), and system output and is crucial for selecting optimal light conditions, especially when multiplexing with other photoreceptors. Therefore, we characterized the forward (i.e., P to P) and reverse (i.e., P to P) action spectra of our engineered BphP1–PpsR2 system. For the forward experiment, we exposed bacteria to 0–20.0 μmol/m²·s light from each of 23 different LEDs with emission wavelengths ranging from 369 to 954 nm. As expected, wavelengths between 740 and 782 nm induce the strongest responses, whereas those between 636 and 677 nm maximally deactivate the system (Figures 5a and S11). Interestingly, the 782 nm response is quantitatively similar to that of 740 and 755 nm (Figure S12), demonstrating robust activation by this highly red-shifted wavelength. Other wavelengths have little effect, though there is some activation in the 388–451 nm region at the higher intensities (Figure 5a). BphP1, like other phytochrome-family photoreceptors, has a small absorption peak in the UV-blue region, also known as the Soret band. As expected, the system is maximally deactivated by 636–677 nm light (Figures S13).

For the reverse action spectrum, we applied sufficient NIR to activate the system to an intermediate level and then performed the same spectral experiment (Figure 5b). As expected, the system is maximally deactivated by 636–677 nm light (Figures S13).
Figure 5. BphP1–PpsR2 action spectra. (A) Forward action spectrum. LED centroid wavelength is shown. (B) Reverse action spectrum. Cells were treated with 7.82 μmol/m²·s 755 nm light to activate the system to an intermediate level and simultaneously exposed to the LED centroid wavelengths shown. All values represent the mean of n = 3 experiments conducted on separate days. The 369 nm LED is incapable of reaching the brightest intensities (gray squares). Absolute sfGFP responses are shown in Figure S11. LED details are listed in Table S5.

5b and S11). Overall, both action spectrum measurements are highly consistent with in vitro BphP1 absorption and action spectra.

## CONCLUSIONS

Our engineered BphP1–PpsR2 system is the most red-shifted bacterial optogenetic tool yet reported. Due to its unique action spectrum and rapid response dynamics, BphP1–PpsR2 should be able to be combined with existing UV, blue, green sensors to achieve independent control of the expression dynamics of multiple genes in the same cell. Such multiplexed optogenetic programming of gene expression dynamics promises to be a powerful new method for revealing new aspects of how information flows through gene networks. Additionally, BphP1–PpsR2 should be able to be controlled while imaging most commonly used fluorescent protein reporters, including infrared fluorescent proteins, a benefit that will facilitate single cell time lapse experiments.

The major limitation to our system is its relatively low (~2.5-fold) dynamic range. However, we previously engineered an E. coli green light sensor with only 2-fold dynamic range that was subsequently used to reveal novel input/output dynamics of a synthetic transcriptional inverter circuit, modulate growth rate via programmed expression of a methionine biosynthetic gene, and demonstrate that synthetic genetic circuits can adaptively filter noise. Thus, even without further optimization, we believe that BphP1–PpsR2 can be used for a range of optogenetic applications.

Nonetheless, the utility of BphP1–PpsR2 should be able to be improved by increasing its dynamic range in future work. Because the PBr_Fe output promoter has a relatively large dynamic range of nearly 80-fold (Figure 1c), we hypothesize that this small response is due to intrinsically small changes in the BphP1 PBr/PFe ratio in response to NIR versus red light. On the other hand, BphP1–PpsR2 has recently been combined with TetR DNA binding and VP16 transcriptional activation domains along with a minimal CMV output promoter containing seven tetO operator sites to achieve ~40-fold NIR activation in mammalian cells. Thus, we believe that the dynamic range of our system could be improved by adding bacterial signal-amplifying genetic modules. Available E. coli transcriptional inverters, RNA-based transcription regulators, viral protease systems, and recombinases that activate strong output promoters have been shown to amplify transcriptional responses by 1–3 orders of magnitude. Alternatively, directed evolution could be performed on BphP1 to increase its photoswitchability.

Other NIR photoreceptors could also be explored. Lagarias and co-workers have recently discovered cyanobacteriochrome sensor histidine kinases (CBCR SKs) that bind phycocyanobilin (PCB) instead of BV, yet absorb strongly in the NIR spectrum up to λmax = 740 nm in the ground state. The major challenge is that these photoreceptors lack known partner response regulators and/or output promoters that are needed to control gene expression. However, domain swapping could potentially be used to replace the PCD of our related green light-sensing CBCR SK CcaS with that from one of these NIR sensors. Ideally, the >100-fold dynamic range and rapid response dynamics of our CcaS-based system would be preserved, resulting in a new NIR sensor that retains the benefits of BphP1–PpsR2 while finding additional utility due to the larger response.

## METHODS

### Plasmids, Strains, and Media.

All plasmids were constructed using Golden Gate or Gibson assembly. The genes encoding PpsR2 and BphP1 (rpa1536 and rpa1537, respectively) were amplified from Rps. palustris CGA009 genomic DNA (ATCC), and the frameshift mutation in rpa1537 was fixed via insertion of a guanine residue at position 430. pEO100c and pSR346 were used as template vectors for building plasmids pNO1–10 and pNO41, respectively. To build pNO1S, we replaced sfGFP downstream of the PBr promoter on pSR11-2 with ppsR2. We replaced mCherry markers with mTurquoise2 or mEos2.
downstream of the P1.1Z0.5 promoter on pNO15 with the corrected bphP1 gene to build pNO17. All synthetic ribosome binding sites (sRBSs) were designed using DeNovoDNA software. To build the ppsR2–bphP1 bicistronic operon plasmids pNO131–194, we computationally designed a set of eight sRBSs with predicted strengths varying over ~57-fold (Table S1) each for ppsR2 and bphP1 and then assembled the 64 possible combinations of sRBS strengths for the 2 genes using pSR58.6 as a template vector. The apFAB30 constitutive promoter controls operon expression on pNO131–194. Cloning was carried out using NEB 10-β cells (New England Biolabs, catalog no. C3019H). Primers were ordered from Integrated DNA Technologies, Inc., IA, and DNA sequencing services from Genewiz or Lone Star Laboratories were used.

All experiments were performed in E. coli BW29655 (BW28357 Δ(envZ-ompR)520 (::FRT)). LB media was used to culture transformed bacteria at 37 °C, 250 rpm, with appropriate antibiotics added (50 μg/mL ampicillin, 34 μg/mL chloramphenicol, and/or 100 μg/mL spectinomycin). All experiments were conducted using M9 media (1x M9 salts, 0.4% w/v glucose, 0.2% w/v casamino acids, 2 mM MgSO4, 100 μM CaCl2) supplemented with appropriate antibiotics listed earlier. LB cultures of transformed bacteria were grown to exponential phase (OD600 < 0.3), diluted 1:1000 in M9 media, and allowed to grow to exponential phase. By keeping the cells growing in exponential phase instead of reaching stationary phase, we minimized any potential physiological changes to the cells. The OD600 of each M9 culture was recorded prior to the addition of filter-sterilized glycerol to a final concentration of 30% (v/v); cultures were then transferred into PCR tubes in 100 μL aliquots and frozen at −80 °C. All experiments described here were started from such individual aliquots of culture frozen at exponential phase. In addition to being convenient, we have previously determined that this protocol reduces day-to-day variability in the outputs of engineered genetic systems.

**Optical Hardware.** All optogenetic experiments were performed in six 24-well Light Plate Apparatus (LPA) devices mounted in a shaking incubator operating at 37 °C, 250 rpm, allowing for precise optical control with up to two LED inputs per culture. LEDs were calibrated using a spectrophotometer (StellarNet UVN-SR-25 LT16) following our previous method. Replicates of each experimental data point were randomized to different well positions where possible to minimize systematic errors from well-specific effects such as uneven LED calibration. Unless otherwise stated, NIR and R illumination used were set at 72 μmol/m2/s for 760 nm and 40 μmol/m2/s for 660 nm.

**Experimental Protocols.** All experiments were performed according to our previous protocol, which we summarize here. Frozen aliquots of engineered cells were thawed at the bench and inoculated at low densities (OD600 = 2 × 10−5 to 1 × 10−4) into fresh media. Inoculation densities were picked on the basis that cell cultures would reach steady state and remain in exponential phase (OD600 < 0.3) by the end of the experiment, thus ensuring the cells undergo minimal physiological changes during the experiment. Chemical inducers anhydrotetracycline (aTc) (Clontech cat. no. 631310) and isopropyl β-D-1-thiogalactopyranoside (IPTG) (VWR cat. no. 14213-263) were added as needed. 500 μL of each inoculated M9 culture was then added into a well of a clear-bottomed black-walled 24-well plate (ArcticWhite AWLS-303008) that was then sealed with adhesive foil (VWR 60941-126). Culture plates were mounted onto preconfigured LPA devices running the selected light exposure program. For dynamics experiments, cells were preconditioned for 4 h under selected light conditions and then switched to the final light conditions using a previously described staggered-start protocol over the next 4 h. The staggered-start protocol allows ease of capturing light dynamics data with precise control of time points while eliminating the need for repeated samplings of a continuous culture. Assays were run for 5 h (PpsR2-promoter screen) or 8 h (optogenetic experiments). At the end of each experiment, the plates were removed from the incubator and placed in an ice–water slurry for 15 min to inhibit further cell growth. 100 μL cell sample aliquots were transferred from each well into prechilled flow cytometry tubes holding 1 mL PBS + 500 μg/mL rifampicin, and these were placed in a 37 °C water bath for 1 h to allow for maturation of translated sfGFP while inhibiting new sfGFP transcription. They were then placed back in ice–water for 15 min and then measured for sfGFP fluorescence using flow cytometry.

**Flow Cytometry and Data Analysis.** Single cell fluorescence was measured using a BD FACSscan flow cytometer as previously described. The bead samples (Spherotech RCP-30-5A) in PBS were measured with each day’s experiments. Following data acquisition, raw flow cytometry files were processed using FlowCal. Cell populations were gated by density, and the arbitrary fluorescence units were converted by FlowCal into standardized MEFL values based on the calibration bead samples. The individual arithmetic means of all gated events were calculated and reported as the population mean of each sample.

**Model Fitting.** All Hill function (deactivating form: \( f(I) = b - \frac{a I^\tau}{I^\tau + K_{1/2}} \)) and dynamics model (Figure S10) fits were performed using a nonlinear least-squares curve-fitting algorithm in Prism v7.0a for Mac OSX (GraphPad Software, Inc., La Jolla, CA). Reported \( t_{1/2} \) values were calculated by addition of the respective fit values of the delay parameter \( t \) and half-life of the exponential parameter \( k \). Because the four function parameters including \( k \) and \( t \) (Figure S10) were fit locally to each step–response curve, \( k \) and \( t \) fit values have symmetric uncertainties (Table S4). However, \( t_{1/2} \) fit values inherit asymmetrical uncertainties from logarithmic calculation of \( k \) half-life uncertainties. Model function parameters were fit locally to each data set of \( n = 3 \) replicates per data point unless otherwise stated.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.7b00289.

**Author Contributions**

N.T.O., E.J.O., and J.J.T conceived of the study and designed the experiments. N.T.O. performed the experiments and

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analyzed the data. N.T.O. and J.J.T wrote the manuscript. All authors discussed the project and commented on the manuscript.

Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS

NIR, near-infrared; BphPs, bacteriophytochrome photoreceptors; PCD, photosensory core domain; BV, biliverdin IXα; MEFl, molecules of equivalent fluorescence; TCS, two-component system; CBCR, cyanobacteriochrome; SK, sensor kinase

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