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A single-component light sensor system allows highly tunable and direct activation of gene expression in bacterial cells

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ABSTRACT

Light-regulated modules offer unprecedented new ways to control cellular behaviour with precise spatial and temporal resolution. Among a variety of bacterial light-switchable gene expression systems, single-component systems consisting of single transcription factors would be more useful due to the advantages of speed, simplicity, and versatility. In the present study, we developed a single-component light-activated bacterial gene expression system (eLightOn) based on a novel LOV domain from *Rhodobacter sphaeroides* (RsLOV). The eLightOn system showed significant improvements over the existing single-component bacterial light-activated expression systems, with benefits including a high ON/OFF ratio of >500-fold, a high activation level, fast activation kinetics, and/or good adaptability. Additionally, the induction characteristics, including regulatory windows, activation kinetics and light sensitivities, were highly tunable by altering the expression level of LexRO. We demonstrated the usefulness of the eLightOn system in regulating cell division and swimming by controlling the expression of the FtsZ and CheZ genes, respectively, as well as constructing synthetic Boolean logic gates using light and arabinose as the two inputs. Taken together, our data indicate that the eLightOn system is a robust and highly tunable tool for quantitative and spatiotemporal control of bacterial gene expression.

INTRODUCTION

Precisely coordinated analogue, temporal and spatial gene expression patterns are required for deciphering complex biological processes in living cells (1,2). Light is a very attractive trigger that, unlike classical small chemicals, can be controlled with millisecond and submicron resolutions. Several light-inducible gene expression systems have been developed for bacteria in recent years. Many of these systems are based on bacterial two-component regulatory systems (TCSs) (3–9). In these systems, light-regulated kinases phosphorylate their cognate response regulators, which in turn drive gene expression from a specific promoter. Thus, these systems need at least two components. Additionally, some TCS-based systems require extraneous chromophores or appropriate chromophore synthesis genes (4–9). Furthermore, many of these systems suffer from low induction ratios (<100-fold) and poor adaptability (specific cognate response regulators to specific promoters).

A single-component bacterial light-switchable gene expression system has the advantage of simplicity, as it consists of a single transcription factor. In 2016, our group reported the first single-component light-inducible system based on the Vivid domain in *Escherichia coli (E.coli)*, termed the LightOff system (10). The system has a high activation level (comparable with that of the T7 system) and require no extraneous chromophore. However, gene expression is repressed upon light illumination in the LightOff system. Although it is possible to switch the system from 'light-off' to 'light-on' using a cI/P_R circuit, this practice would not only increase the complexity but also cause a delayed ON/OFF switch, as accumulation or degradation of the cI repressor requires time. In 2016, Jayaraman *et al.* de-

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veloped a light-inducible gene expression system based on a single light-sensitive repressor derived from the EL222 protein, which allows direct activation of gene expression in *E. coli* by light (11). However, the system suffered from an extremely low ON/OFF ratio (<5-fold), which is impractical for many biological studies and biotechnical applications.

The most promising light-inducible system should be simple, easy to manipulate and independent extraneous chromophores and have a high activation level and induction ratio (12). In the present study, we developed a single-component light-activated gene expression system, termed the eLightOn system, which has a distinct light-regulated behaviour compared to that of the LightOff system based on LEVI. We first created a synthetic light-switchable repressor, LexRO, based on a novel LOV light sensor domain, RsLOV. In darkness, LexRO dimerizes and binds to its cognate operator sequence to repress promoter activity. Upon light exposure, the LexRO dimer dissociates, causing dissociation from the operator sequence, and initiates gene expression. The eLightOn system showed significant improvements over the existing single-component bacterial light-activated expression systems, with benefits including a high ON/OFF ratio of >500-fold, a high activation level, fast activation kinetics, and/or good adaptability to different promoters and *E. coli* strains. We also obtained a multitude of regulatory systems with highly tunable induction characteristics, including background noise, maximal activation levels, activation kinetics and light sensitivities. Further studies showed that the eLightOn system could be utilized to control bacterial swimming and division by controlling the expression of CheZ and FtsZ, respectively. We also utilized LexRO to construct several Boolean logic gates using light and arabinose as the two inputs. Taken together, our data indicate that the eLightOn system is a robust and versatile tool for rapid, reversible, quantitative and spatiotemporal control of gene expression in bacteria.

MATERIALS AND METHODS

DNA cloning

Unless stated otherwise, cloning was performed using the Hieff Clone™ One Step Cloning Kit (YEASEN). The *RsLOV* gene was synthesized by Wuxi Qinglan Biotech Co., Ltd and inserted into pLEVI$_{408}$-mCherry (a plasmid from the LightOff system containing the light-switchable repressor LEVI$_{408}$ and P_{ColE} promoter-driven expression of the mCherry reporter gene (10), where LEVI$_{408}$ consists of the Vivid light-sensing domain and DNA-binding domain of LexA$_{408}$ (a mutant of LexA that recognizes a symmetrically altered operator mutant but not wild-type operator) (13,14)) to replace the *Vivid* gene to obtain the original vector pLexRO-mCherry (ori). Primers containing 1–8 amino acid random linker-encoding sequences were used to amplify the above original vector to obtain a plasmid pool for screening linkers between the LexA$_{408}$ and RsLOV domains. Primers containing different Shine–Dalgarno (SD) sequences upstream of the *LexRO*-encoding gene were used to amplify pLexRO-mCherry, and the linearized fragments were phosphorylated and ligated to obtain pLexRO-mCherry (SDs). Plasmids containing constitutive promoters (J23106, J23107, J23116, J23114 and J23117) with varied activity-driven expression of LexRO were obtained using a similar strategy. The *CheZ* and *FtsZ* genes were amplified from the genome of *E. coli* and inserted into pLexRO-mCherry to obtain pLexRO-CheZ (SD2) and pLexRO-FtsZ (SD17), respectively. The *mCherry* gene fragment was amplified from pLEVI$_{408}$-mCherry and inserted into BamHI and XhoI sites in the pDawn vector (Addgene: #43796) to obtain pDawn-mCherry. The F30-2xdBroccoli fragment was amplified from pET28c-F30-2xdBroccoli (Addgene: #66843) and inserted into pLexRO-mCherry (SD2/17/37), pDawn-mCherry and pLEVIon-mCherry to obtain pLexRO-F30-2xdBroccoli (SD2/17/37), pDawn-F30-2xdBroccoli and pLEVIon-F30-2xdBroccoli, respectively. The *cI* repressor gene was amplified from pLEVIon-mCherry and inserted into pLexRO-mCherry (SD2) to obtain pLexRO-cI. The P_R promoter sequence was amplified from pLEVIon-mCherry, fused with the mCherry gene using overlap PCR, and then inserted into BglII and XhoI sites in the pET28a vector to obtain pET28a-P$_R$-mCherry. An *AraC* expression cassette was amplified from pBAD/His and inserted into pLexRO-mCherry (SD17) to replace the *LacI* expression cassette, and then the P_{BAD} promoter sequence was inserted upstream of the ColE$_{408}$ promoter to obtain pLexRO-P$_{BAD}$-ColE$_{408}$-mCherry for an 'OR' gate. The *LasI* gene fragment was amplified from the genome of *Pseudomonas aeruginosa* and inserted into the NcoI and HindIII sites in the pBAD/His vector to obtain pBAD-LasI. The *LasR* gene fragment was amplified from the genome of *P. aeruginosa* and inserted into pLexRO-mCherry (SD2) to obtain pLexRO-LasR (SD2). The P_{LasI} promoter was synthesized by Shanghai Generay Biotech Co., Ltd, fused with *mCherry* and inserted into HindIII and XhoI sites in the pET28a vector to obtain pET28a-P$_{LasI}$-mCherry. The *cI* gene was fused to the P_{LasI} promoter and rrnB transcription terminator sequences using overlap PCR to obtain P$_{LasI}$-cI-rrnB and then inserted into pET28a-P$_R$-mCherry to obtain pET28a-P$_{LasI}$-cI-P$_R$-mCherry. The *LasR* gene was fused to the P_R promoter and rrnB transcription terminator sequences using overlap PCR to obtain P$_R$-LasR-rrnB and was inserted into pET28a-P$_{LasI}$-mCherry to obtain pET28a-P$_{LasI}$-mCherry-P$_R$-LasR. The pLexRO-mCherry (SD2/3/7/17/37) vectors were linearized using reverse PCR to introduce a Flag tag-encoding sequence at the C-terminus of the LexRO gene. The linearized sequences were phosphatized and ligated to obtain pLexRO-Flag-mCherry (SD2/3/7/17/37). The pLexRO-mCherry (SD17) vector was linearized using reverse PCR to introduce the tac, lac, J23110 and T7 promoter sequences to replace the ColE$_{408}$ promoter. The linearized sequences were phosphatized and ligated to obtain pLexRO-tac-mCherry, pLexRO-lac-mCherry, pLexRO-J23110-mCherry and pLexRO-T7-mCherry, respectively. All these materials are available upon request.

Cell growth and blue light irradiation

Unless stated otherwise, all experiments were carried out in the *TOP10 E. coli* strain. eLightOn systems used in the study are shown in Supplementary Table S1. The cells were cultured in LB medium with 50 $\mu g \cdot ml^{-1}$ spectinomycin and

grown at 37°C with shaking at 230 rpm. For detection of light-regulated gene expression, cells were cultured in 48-well plates and illuminated by 3 $mW{\cdot}cm^{-2}$ blue light emitting from an LED lamp (460 nm peak) or remained in the dark before characterization. Neutral density filters were used to adjust the light intensity. Light intensities were measured with a luminometer (Sanwa, LX-2). For dark manipulation, a red (620–630 nm) LED lamp was used. Induction of protein expression from the pBAD system in the *TOP10* strain and the pET and pCDFDuet1 systems in the *BL21(DE3)* strain was carried out by adding 0.2% arabinose and 1 mM IPTG, respectively. To detect the performance of different genetic circuits, the plasmids were co-transformed into the *TOP10* strain. Unless otherwise indicated, mCherry fluorescence was determined 15 h after the cells were cultured with or without the inducers. mCherry fluorescence and OD_{600} were measured by a Synergy 2 multimode microplate reader (BioTek) (ex = 590/20 nm, em = 645/40 nm). The fluorescence was normalized to the OD_{600} of each sample.

To test the activation kinetics of the eLightOn system, overnight cultured cells were diluted to OD_{600} ≈0.001 and grown under noninducing conditions. Cells were transferred to blue light illumination until $OD_{600} \approx 0.1$. For mCherry protein measurement, aliquots were taken at the indicated time points, and cell growth was arrested by the addition of 3.3 $mg{\cdot}ml^{-1}$ chloramphenicol and 0.4 $mg{\cdot}ml^{-1}$ tetracycline. The cultures were incubated in an ice-water bath for 10 min to rapidly stop gene expression and were then incubated in a 37°C water bath for 1 h (3,15). For Broccoli RNA measurement, aliquots were taken at the indicated time points, and the green fluorescence (Broccoli-DFHBI-1T complex) at the indicated time points after light illumination was measured by incubating the cells with 20 μM DFHBI-1T (Lucerna) in buffer containing 40 mM HEPES, pH 7.4, 125 mM KCl and 5 mM $MgCl_2$. The cultures were measured using a CytoFLEX-S flow cytometer (Beckman Coulter).

To examine the ability of the eLightOn system to spatially control gene expression, *TOP10* cells transformed with pLexRO-mCherry were cultured overnight under non-inducing conditions. The cells were mixed with LB medium containing 1% agar, and the final OD_{600} was ∼0.1 (it was ensured that the temperature of the LB medium was below 50°C). The mixture was immediately poured into a Petri dish with a 90-mm internal diameter and allowed to harden at room temperature for 30 min. The dish was covered by a photomask and incubated at 37°C for 15 h with blue light (1.57 $mW{\cdot}cm^{-2}$) illuminating the agar-embedded film of bacteria expressing mCherry protein. A Multispectral System FX (Carestream Health) with excitation and emission filters of 600/20 nm and 670/50 nm, respectively, was used. mCherry expression by the cells on the plate was quantified by the ImageJ image-processing program.

Imaging and flow cytometry

For fluorescence imaging, images were acquired using a Leica SP8 confocal laser scanning microscope equipped with an HC PL APO CS2 63.0× 1.40 Oil objective, using an excitation wavelength of 561 nm and an emission range of 570–670 nm for mCherry.

A BD FACSJazz™ flow cytometer with a 561-nm laser was used for sorting. To analyse the mCherry fluorescence produced by bacteria, a CytoFLEX-S flow cytometer (Beckman Coulter) with an excitation wavelength of 561/10 nm and an emission wavelength of 610/20 nm was used. After acquisition, the raw cytometry data were processed and analysed using the Cytexpert program (Beckman Coulter).

Western blot analysis

Equal amounts of the total lysate protein (20 μg) were electrophoresed on a 12% SDS-PAGE gel and then transferred to polyvinylidene fluoride (PVDF) membranes (PALL). Membranes were blocked with 0.5% casein and then probed with primary antibodies (mouse anti-Flag tag mAb (1:500; Santa Cruz SC-166355) and mouse anti-GAPDH mAb (1:2000; Arigo ARG62345)). Subsequently, the membranes were treated with secondary antibodies (m-lgGκ BP-HRP for anti-Flag (1:2000; Santa Cruz SC-516102), goat anti-mouse lgG conjugated to HRP for anti-GAPDH (1:5000, Arigo ARG65350)). Immunoreactivity was detected using a BM Chemiluminescence Blotting Kit (Roche Diagnostics) according to the manufacturer's protocol with a Tanon-5200 Multi imager.

Light-controlled bacterial mobility

Light-controlled cell mobility was assayed with *JM109(DE3, ΔsulA, ΔLexA, ΔcheZ)* cells, whose cheZ genes were knocked out based on *JM109(DE3, ΔsulA, ΔLexA)* using the λ Red recombination system. Light-controlled cell mobility was assessed on semisolid agar. To prepare semisolid agar, 10 ml of LB medium containing 2.5 g of yeast extract, 5 g of Bacto tryptone, 5 g of NaCl and 0.5% (w/v) glycerol per liter supplemented with 0.25% agar (Difco, Bacto agar) was poured into a Petri dish with a 6-cm internal diameter and allowed to harden at room temperature for 90 min. Engineered bacteria transformed with pLexRO-cheZ were picked into fresh LB and cultured at 37°C. When the OD_{600} reached ∼0.2, a suspension of cells (2 μl) was spotted onto the semisolid agar plate and cultured under differing light irradiance. All experiments were carried out at 37°C. Images of bacterial mobility were acquired using a Kodak In-Vivo Multispectral System FX (Carestream Health).

Light-controlled bacterial division

Light-controlled cell division was assayed in *TOP10 (ΔftsZ)* cells, whose ftsZ gene was knocked out following transformation of *TOP10* cells with pLexRO-ftsZ using the λ Red recombination system. The engineered bacterial cells were cultured with blue light illumination (0.9 $mW{\cdot}cm^{-2}$) until $OD_{600} \approx 0.1$ and then transferred to dark conditions. The cells were collected at the indicated time points, and image data acquisition was performed using a Leica SP8 confocal laser scanning microscope.

RESULTS

Development of a synthetic light-switchable transcription factor

We previously reported several light-switchable gene expression systems based on single-component transcription factors for mammalian cells, bacteria and yeast (10,16–18). All the light-switchable transcription factors utilized the LOV domain Vivid (VVD) from *Neurospora crassa* as the light-sensing and responding domain. For the bacterial LightOff system, upon exposure to blue light, the VVD domain dimerizes and promotes the transcription factor LEVI binding to DNA sequences to directly repress gene transcription (10). We hypothesized that replacing the VVD domain in LEVI with a photosensitive domain harboring contrasting light-inducible properties to VVD might create a light-activated system for bacteria. RsLOV is a blue light sensor from *Rhodobacter sphaeroides* that possesses a contrary light-inducible behavior to VVD (19) and has been used to engineer temperature- and light-switchable Cas9 variants (20). To this end, we fused RsLOV to the C-terminus of the DNA-binding domain of the $LexA_{408}$ repressor (a mutant of LexA that recognizes a symmetrically altered operator mutant but not a wild-type operator) (13,14). In darkness, RsLOV dimerizes and causes subsequent dimerization of the fusion protein, which in turn binds its cognate operator sequence and represses promoter activity (Figure 1A). Upon blue light exposure, light-induced formation of a flavin-cysteinyl photoadduct disrupts hydrogen bonding in the active site and propagates structural changes through the LOV domain core to the N- and C-terminal extensions, which causes the RsLOV dimer to dissociate into monomers and results in DNA dissociation, initiating gene expression (Figure 1A).

We constructed a plasmid pool containing random linkers with different lengths (1–8 amino acids) and amino acids between the RsLOV and $LexA_{408}$ domains. The plasmid pool was transformed into the *E. coli TOP10* strain and first cultured under blue light illumination (Figure 1B). The cultures were analyzed by flow cytometry, and the cells showing relatively high mCherry signals were sorted. The cells were then cultured under dark conditions, and the cells with relatively low mCherry signals were sorted (Figure 1B). This alternating light-dark screening procedure was performed three times. The sorted cells from the last round were plated and cultured under light conditions. Hundreds of clones with red fluorescence were picked and further verified (Supplementary Figure S1). Several mutants manifesting >50-fold activation of mCherry expression were sequenced (Figure 1C, Supplementary Figure S1). We referred to $LexA_{408}$-RsLOV with the linker 26R (KV), which showed the highest activation ratio (>500-fold), as LexRO and the single-component light-activated system based on LexRO as the eLightOn system.

The eLightOn system consists of a single-component light-switchable transcription factor, which is simple and reduces the possibility of interaction with the host chassis itself. The eLightOn system with SD17 showed a significantly higher activation level than the pDawn and pLEVIon systems, yet with lower leakage than the pDawn and T7-driven expression system under noninducing conditions (Supplementary Figure S2), leading to a much higher induction ratio (Supplementary Figure S2). The eLightOn system could also control gene expression in commonly used *E. coli* strains (Figure 1D). In addition, the light-switchable transcription factor LexRO could be used to control the transcriptional activities of various promoters in which the $LexA_{408}$ cognate operator sequence was incorporated (Figure 1E). These data demonstrate that the eLightOn system has good adaptability to different strains and promoters.

Fine tuning the regulatory dynamics of the eLightOn system

We next tested the effects of the expression levels of LexRO on the performance of the eLightOn system by randomly altering the SD sequence upstream of the LexRO-encoding gene. We obtained a multitude of regulatory systems in which background noise and maximal activation levels were highly diverse (Figure 2A–C, Supplementary Table S2), which would be useful for specific experimental conditions when different regulatory windows are required. We next chose eLightOn systems with SD2, 3, 7, 17 and 37 to test the content of intracellular LexRO. Our results showed that LexRO expression levels differed markedly among these systems. eLightOn systems with higher background noise showed lower LexRO expression, while lower background noise was associated with much higher LexRO expression (Supplementary Figure S3). Similar results were also observed for LexRO expressed from constitutive promoters with varied activities (Supplementary Figure S4). It seemed that high LexRO expression resulted in homodimer formation independent of light illumination, which repressed promoter activities, even under light conditions. In contrast, low LexRO expression could not form sufficient amounts of homodimer to repress the activities of the promoter, enabling high background noise under dark conditions. In fact, a similar effect of the regulator expression level on background activity was also observed for the LightOn system we developed previously (16,21). The GAVPO light switch in the LightOn system is a transcriptional activator, whereas LexRO is a repressor. Therefore, the correlations between expression levels of the light switch proteins and background activities of these two systems seem to be apparently 'opposite'. Overall, the regulatory windows of the eLightOn system are highly tunable by altering the expression levels of LexRO.

Characteristics of the light-activated systems

We sought to investigate the activation kinetics of the eLightOn system. The cells were first cultured under dark conditions and then transferred to light illumination. Our results showed that eLightOn systems with differing LexRO expression exhibited varied activation kinetics. The eLightOn system with SD37 showed the fastest mCherry reporter expression activation kinetics, with a $t_{1/2}$ (the time to reach 50% of maximal expression) of 84 min, which was significantly faster than those of the eLightOn systems with SD2 and SD17 ($t_{1/2} \sim$ 156 min and 112 min, respectively) and the pDawn and pLEVIon systems ($t_{1/2} \sim$133 min and 346 min, respectively) (Figure 3A, B, Supplementary Fig-

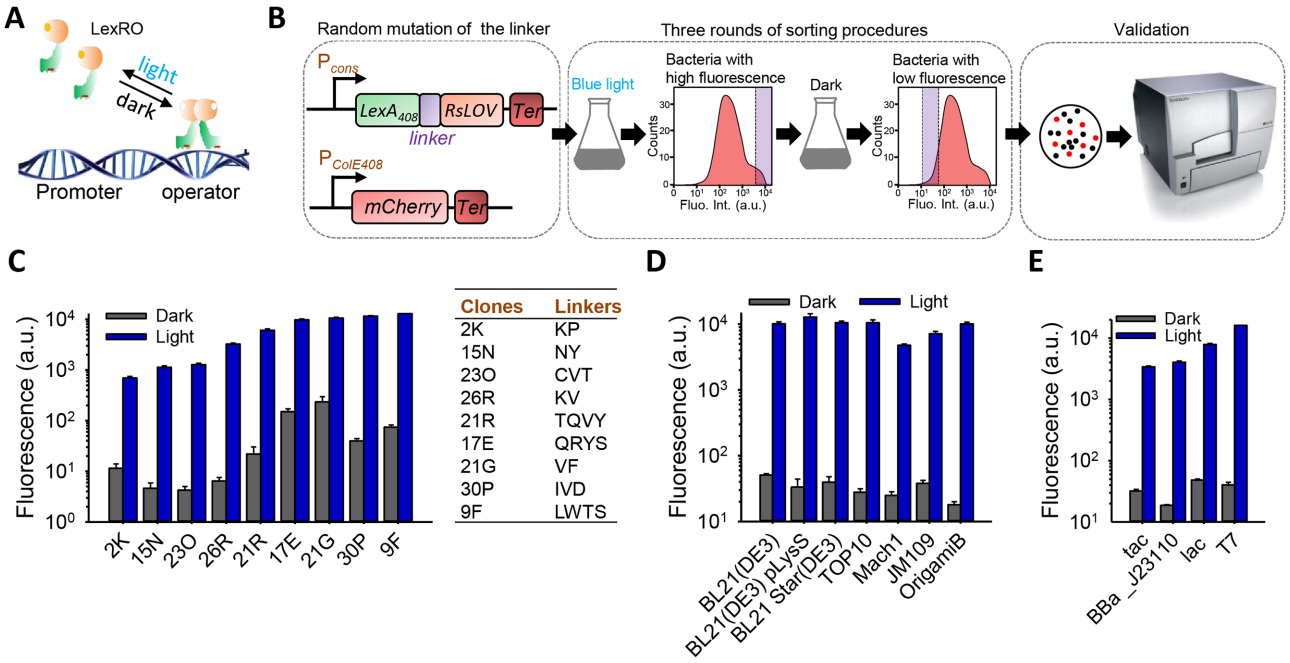

Figure 1. Development of a single-component light-activated gene expression system for bacteria. (**A**) Schematic representation of the eLightOn system. Under dark conditions, the light-switchable repressor dimerizes and binds its cognate operator sequence to repress promoter activity. Light illumination results in gradual dissociation of the dimers and transcription activation. (**B**) Screening of the light-switchable repressor by FACS. (**C**) Validation of the linkers. The clones harboring $LexA_{408}$-RsLOV variants showing >50 ON/OFF ratios were further validated, and the linkers were sequenced. (**D**) Controlling gene expression in various *E. coli* strains using the eLightOn system by light. The eLightOn system was transformed into different *E. coli* strains. mCherry expression under light or dark conditions was measured. (**E**) Adaptability of LexRO in controlling transcriptional activities of various promoters. The $LexA_{408}$ cognate operator sequence was incorporated into various constitutive promoters. Detection of the activities of these promoters upon blue light irradiance or in darkness was conducted in *TOP10* cells. Data shown in all bar graphs represent the mean ± SD from three independent transformants. a.u., arbitrary units.

ures S5A and S6). We then tested the RNA levels activated by these systems by using Broccoli, an RNA aptamer mimic of GFP (22), as the reporter (Supplementary Figure S5B). The results showed that the RNA accumulation rates were SD37>SD17>pDawn>SD2>pLEVIon, which was consistent with the protein data (Figure 3C). Notably, the pDawn system (cI-LAA/P_R) had faster activation kinetics than the pLEVIon system (cI/P_R), probably because fusion of the LAA tag could accelerate the degradation of the cI repressor, which in turn relieved the repression on the activity of the P_R promoter. In addition, strong dose dependence of the duration of blue-light pulses was observed for the eLightOn system (Supplementary Figure S7); even a 30-min pulse of blue light led to 2.0-, 11.2- and 1.5-fold activation of mCherry reporter expression for SD2, SD17 and SD37, respectively. Thus, activation levels controlled by the eLightOn system can be continuously adjusted by either light intensity or duration of the illumination pulse.

We further tested the light intensity-dependent activation of the eLightOn system. Cells were exposed to light illumination at different wavelengths and light intensities, and mCherry fluorescence was measured. We found that the eLightOn system was highly sensitive to blue light ranging from 440 to 475 nm (Figure 3D), consistent with the photochemical properties of LexRO (Supplementary Figure S8) (19). Notably, the eLightOn system with SD37 exhibited a light sensitivity (half-maximal response, k) of 0.059 mW/cm^2 to 460–465 nm blue light (Supplementary Table S3), which was significantly more sensitive than the eLightOn systems with SD17 and SD2 ($k = 0.406$ and 1.312 mW/cm^2, respectively) (Supplementary Figure S9, Supplementary Table S3), demonstrating that eLightOn systems with lower LexRO content were more sensitive to light illumination. Thus, we provided a novel way to regulate the induction characteristics of an optogenetic system other than altering the light-induced characteristics of the photosensitive domain (10,23).

The ability of the eLightOn system to spatially control gene expression was examined using a plate-based assay, similar to in a previous report (10). Bacterial lawns covered by a specific photomask were exposed to blue light. Subsequent fluorescence imaging showed an mCherry expression pattern that precisely reflected the shape of the photomask (Supplementary Figure S10). These results demonstrated that the eLightOn system is a robust and tunable tool for rapid, quantitative and spatiotemporal control of gene expression in bacterial cells.

Control of bacterial swimming and division by the eLightOn system

Phototactic behaviour exists widely in plants and insects, e.g., most plants and some insects move towards a light source. Chemotaxis exists in some bacteria; cheZ plays an important role in bacterial mobility, as cheZ deletion causes cells to tumble incessantly, resulting in a nonmotile phe-

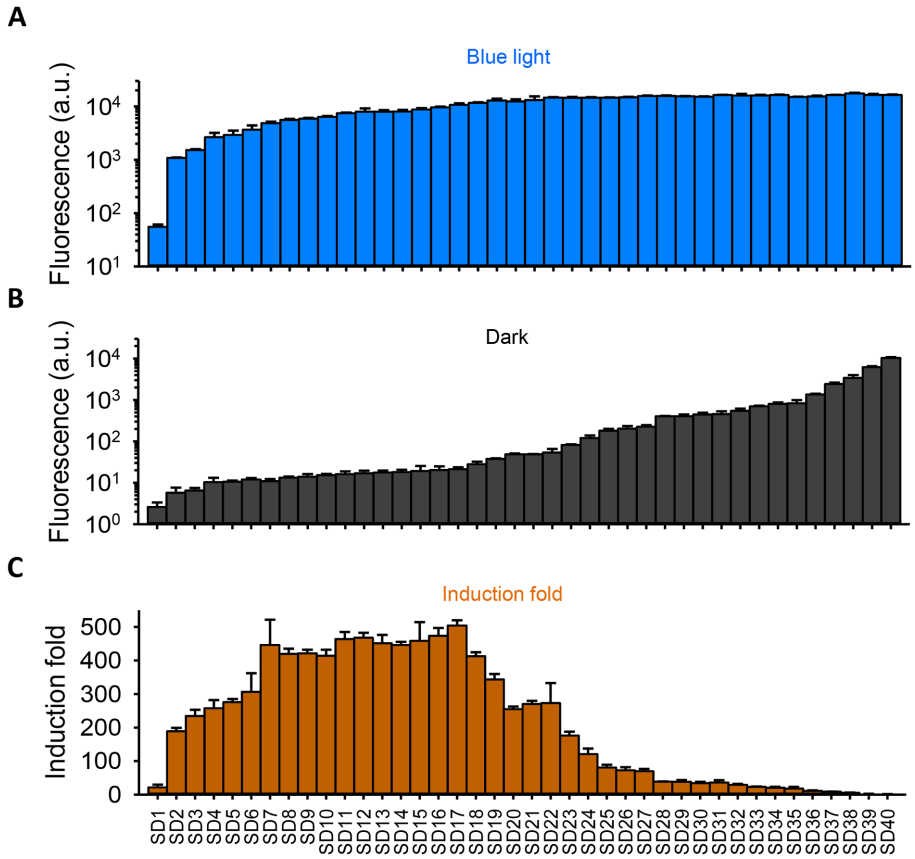

Figure 2. Fine-tuning the regulatory windows of the eLightOn system. The LexRO content of the eLightOn system was altered by changing the SD sequence upstream of LexRO. mCherry expression from eLightOn systems with different SD sequences under light (**A**) or dark (**B**) conditions was measured, and ON/OFF ratios were calculated (**C**). Data represent the mean ± SD from three independent transformants. a.u., arbitrary units.

notype in semisolid agar. Reintroducing cheZ restores cell motility (24). We utilized the eLightOn system to control cheZ expression in a cheZ-deleted strain (Figure 4A). The results showed that bacteria moved upon light illumination and stopped under dark conditions (Figure 4B). The mobility rates of such phototactic bacteria depended on light intensity (Figure 4C), which was probably due to the expression level of the CheZ protein.

The essential cell division protein FtsZ forms a contractile ring structure (Z ring) that controls the timing and location of cell division by recruiting other cell division proteins to the septum to produce a new cell wall between the dividing cells (25). We used the eLightOn system to control FtsZ expression in an FtsZ-deleted strain. Our results showed that *E. coli* cells failed to divide into two separated cells (Figure 4D) but continued to elongate after they were transferred to darkness (Figure 4E). These data demonstrate that the eLightOn system can be used to control diverse cellular activities.

Construction of Boolean logic gates using the eLightOn system

Boolean logic gates integrate multiple digital inputs into a digital output. In living cells, regulatory networks encode logic operations that integrate environmental and cellular signals (26). Diverse synthetic genetic logic gates, including those performing 'AND', 'OR', 'NOT' and 'NOR' functions, have been used in pharmaceutical and biotechnological applications (26,27). However, only a few genetic circuits using light as the input have been reported (5,11,28), although light input may increase the possibility of constructing increasingly complex genetic circuits. In particular, the eLightOn system has highly tunable regulatory windows, enabling it to be an ideal candidate for engineering *E. coli* cells to perform different logic functions using blue light as an input.

A simple NOT gate was obtained by introducing the cI/P_R 'NOT' gate gene circuit (Supplementary Figure S11A), in which the cI repressor gene is placed under the control of the $P_{colE408}$ promoter, and the mCherry reporter is expressed from the cI-repressible P_R promoter. We observed mCherry reporter expression only in darkness (Supplementary Figure S11B), showing converted regulation compared to that of the eLightOn system.

The stepwise construction of an 'AND' gate with arabinose and blue light as the inputs and mCherry as the output is shown in Figure 5A, in which the LasI and LasR genes were placed under the control of the P_{BAD} promoter and $P_{ColE408}$ promoter, respectively, and mCherry was expressed from a second plasmid under the control of the P_{LasI} promoter. The LasI protein is an acyl homoserine lac-

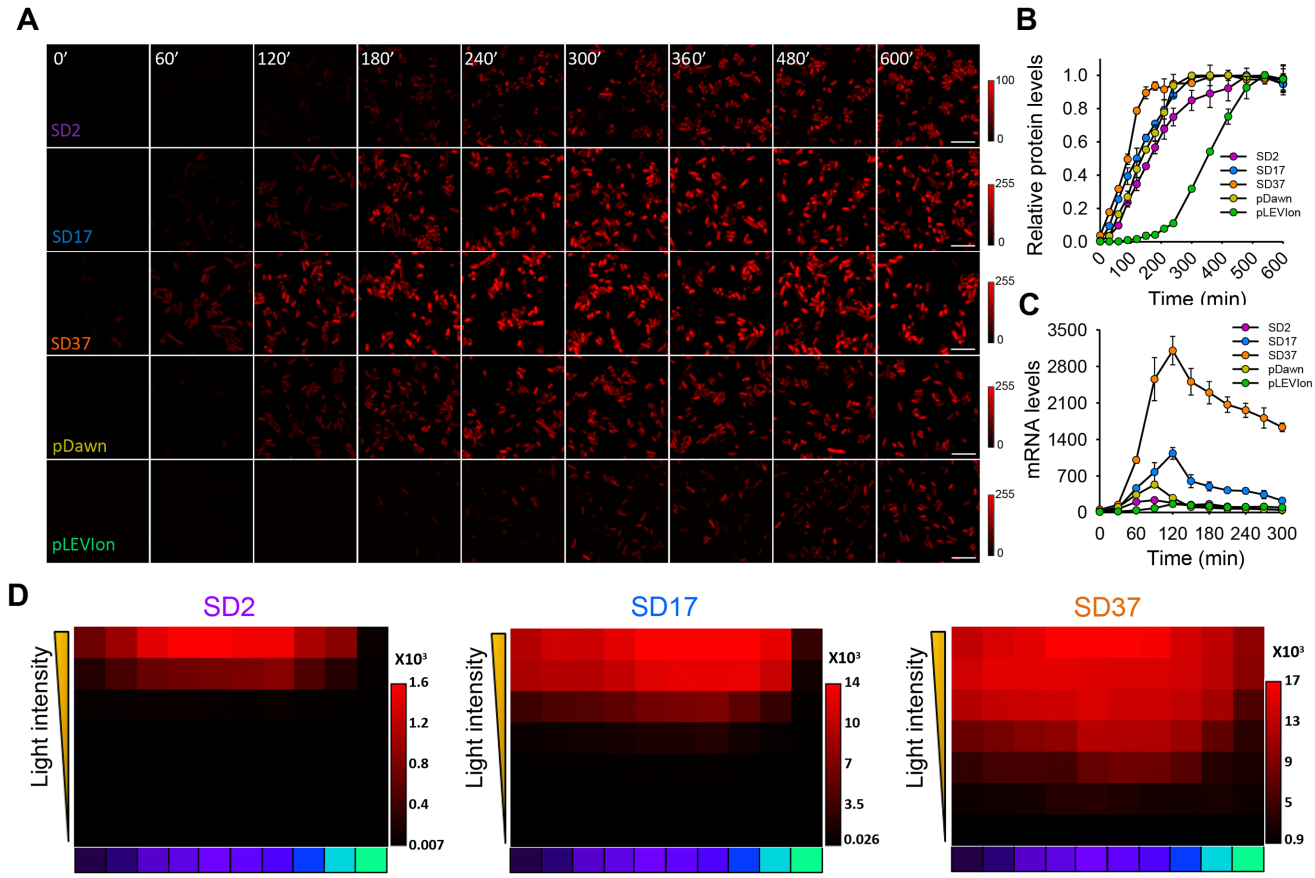

Figure 3. Characterization of the eLightOn system. (A, B) Activation kinetics of mCherry expression by the light-activated gene expression systems in *E. coli*. BL21 (DE3) cells were transformed with eLightOn systems containing different LexRO expression levels (SD2, SD17 and SD37) or pDawn system. JM109(DE3, ΔsulA, ΔLexA) cells were transformed with pLEVIon system. All the systems used mCherry protein as the reporter. The cells were first cultured under dark conditions and then transferred to blue light illumination. mCherry fluorescence at the indicated times was imaged (**A**) and quantified (**B**). Data represent the mean ± SD from three independent transformants. Scale bar, 10 μm. (**C**) Activation kinetics of RNA expression by the light-activated gene expression systems. BL21 Star™ (DE3) cells were transformed with eLightOn systems containing different LexRO content levels (SD2, SD17 and SD37) or pDawn system. JM109(DE3, ΔsulA, ΔLexA) cells were transformed with pLEVIon system. All the systems used F30-2xdBroccoli as the reporter. Green fluorescence (Broccoli-DFHBI-1T complex) at the indicated time points after light illumination was measured by incubating the cells with 20 μM DFHBI-1T in buffer containing 40 mM HEPES, pH 7.4, 125 mM KCl and 5 mM $MgCl_2$. Data represent the mean ± SD from three independent transformants. (**D**) Light irradiance-dependent expression of the eLightOn system. The uninduced cells transformed with eLightOn systems with different SD sequences in dark conditions were diluted into fresh medium and kept in darkness until the OD_{600} reached ≈ 0.1. The cells were then illuminated at the indicated light conditions for 6 h before mCherry fluorescence was measured. The peak spectra from violet to green of the LED lamps were 400–410, 410–420, 420–430, 435–440, 440–445, 450–455, 460–465, 470–475, 490–500 and 520–525 nm, respectively. The light intensities were 0, 0.01, 0.05, 0.15, 0.41, 1.35, 2.79 mW/cm^2, respectively. Data represent three independent transformants.

tone (AHL) synthase. AHL binds to the LasR transcription factor and activates the promoter P_{LasI} (11). Our results showed that the 'AND' gate was nearly digital, achieving 63-, 35- and 34-fold induction between the 'ON' state (light+/arabinose+) and 'OFF' state (light−/arabinose−, light−/arabinose+ and light+/arabinose−, respectively) (Figure 5A, Supplementary Figure S12A).

A 'NAND' gate was easily obtained based on the 'AND' gate: the cI repressor gene was placed under the control of the P_{LasI} promoter, and the mCherry reporter was expressed from a second plasmid under the control of the cI-repressible P_R promoter (Figure 5B). Our data showed that the 'NAND' gate had some characteristics of fuzzy logic, and the highest ratio between the 'ON' state and the 'OFF' state was 10-fold (Figure 5B, Supplementary Figure S12B).

Tandem promoters are common in bacterial genomes (29); however, interference between the promoters may occur when producing an OR function. We constructed an 'OR' gate by placing the P_{BAD} and $P_{ColE408}$ promoters in tandem with the same orientation (Figure 5C). P_{BAD}-$P_{ColE408}$ demonstrated OR logic: either arabinose or blue light could activate mCherry expression, achieving 393-, 148- and 394-fold induction between the 'ON' state (light-/arabinose+, light+/arabinose- and light+/arabinose+, respectively) and 'OFF' state (light−/arabinose−) when one or both inputs was present (Figure 5C, Supplementary Figure S12C). Notably, induction ratios of the $P_{colE408}$ and P_{BAD} promoters in the tandem promoter were lower than those of each of the promoters alone (>500-fold for $P_{colE408}$ and >1000-fold for P_{BAD}(10)), suggesting that the efficiencies of both the $P_{colE408}$ and P_{BAD} promoters were slightly reduced in the tandem promotor.

Finally, we obtained an 'N-IMPLY' gate where the cI and LasI genes were placed under the control of the $P_{ColE408}$

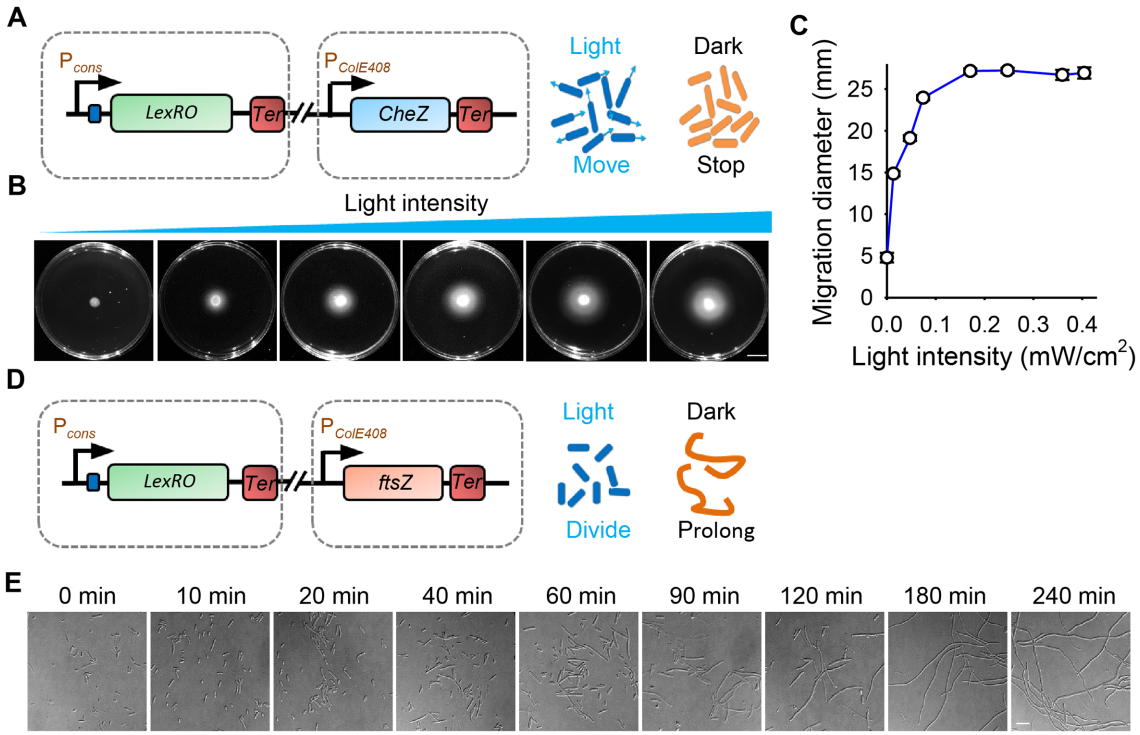

Figure 4. Control of cell swimming and division by eLightOn system. (**A**) Schematic representation of light-controlled cell mobility by the eLightOn system (SD2). (**B**) The engineered bacteria were spotted onto semisolid media and kept under differing light irradiance for 15 h before imaging. Scale bar, 1 cm. (**C**) Calculation of the migration diameter of the cells under differing light irradiance. (**D**) Schematic representation of light-controlled cell division by the eLightOn system. (**E**) An eLightOn system (SD17) using FtsZ as the reporter was transformed into FtsZ-deleted *E. coli* cells. The cells were first cultured under light illumination and then transferred to dark conditions. The morphology of the cells was imaged at the indicated time points. Scale bar, 10 μm.

promoter and P_{BAD} promoter, respectively. The LasR gene was placed under the control of the P_R promoter, and the mCherry reporter gene was expressed from a second plasmid under the control of the P_{LasI} promoter (Figure 5D). The results showed that the mCherry reporter was expressed only under dark conditions with the addition of arabinose (Figure 5D, Supplementary Figure S12D).

Taken together, our data demonstrated that the eLightOn system is a good candidate to construct diverse Boolean logic gates, providing the possibility of obtaining increased complexity logic using light as an input.

DISCUSSION

The emergence of new photosensitive proteins or re-engineering existing photoreceptors has widely extended the applicability of optogenetics, including controlling neuronal excitability (30,31), gene expression (32), and enzyme activities (33) (Optobase, https://www.optobase.org/). In the present study, we used a novel light sensor protein, RsLOV, to develop a light-switchable transcription factor for optogenetic control of gene expression. The RsLOV domain has unique light-inducible behaviour compared to that of the VVD domain, as well as EL222, which we and others have used in previous studies (10,11,16–18,21,34–36). The RsLOV domain dimerizes in the dark but dissociates into monomers upon light excitation. This light-inducible behaviour is in contrast to that of VVD and EL222, for which light causes dimerization. Therefore, the synthetic repressor LexRO based on RsLOV that we built in this study dissociates and loses its repressing activity under light conditions, enabling light-induced gene expression.

The light-activated system based on LexRO displays several advantages over existing light-activated gene expression in *E. coli*. First, the eLightOn system consists of a single-component light sensor, making it very simple and compact. Second, LexRO in the eLightOn system is independent of extraneous photon acceptor or appropriate cofactor synthesis genes, as the cofactor of RsLOV is flavin mononucleotide (FMN), which exists abundantly in bacterial cells. Third, LexRO interacts with the operator sequence of the promoter upstream of the reporter gene and directly activates transcription without a cI/P_R 'NOT' gate gene circuit, which is a straightforward regulation method and significantly accelerates ON/OFF kinetics, as there is a lag for cI accumulation and degradation. Fourth, the eLightOn system possesses highly tunable induction characteristics, including regulatory windows, activation kinetics and light sensitivities, providing flexible options for different experimental conditions. Fifth, the eLightOn system with SD17 has a much higher ON/OFF ratio (>500-fold) than a single-component system based on EL222 (<5-fold) (Supplementary Table S4). Additionally, RsLOV is much smaller than any other dimerization system based on photoreceptors, allowing us to avoid the occurrence of steric hindrance and greatly facilitate accurate molecular design of optogenetic tools. As the first LOV light sensor domain reported to have the characteristics of light-induced dissociation of homod-

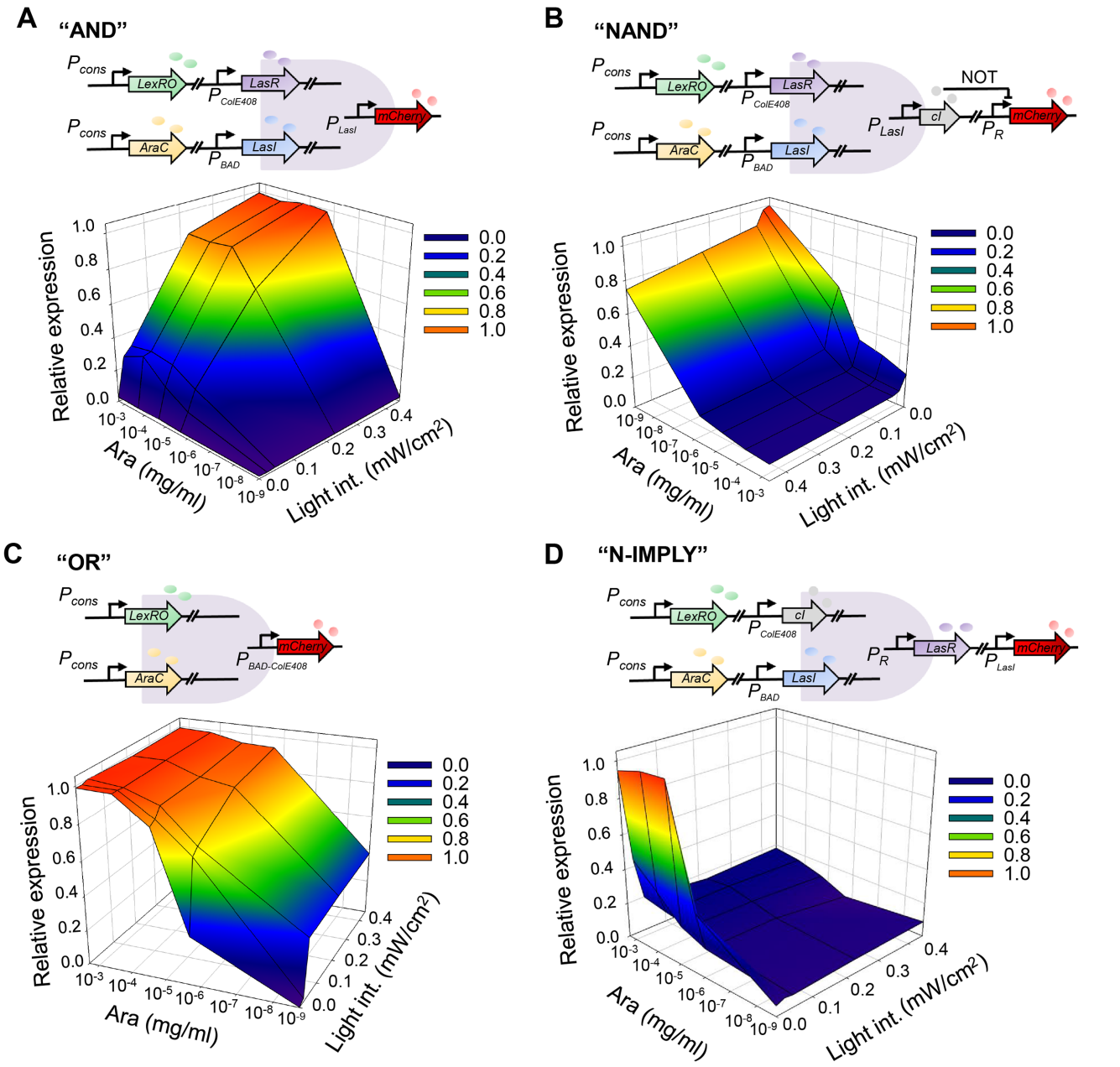

Figure 5. Construction of Boolean logic gates using the eLightOn system. Engineered cells transformed with plasmids performing 'AND' gate (**A**), 'NAND' gate (**B**), 'OR' gate (**C**) and 'N-IMPLY' gate (**D**) logic functions were treated with different inputs. The *TOP10* cells transformed with different plasmids constructs performing 'AND' gate (pLexRO-LasR (SD2), pBAD-LasI and pET28a-PLasI-mCherry), 'NAND' gate (pLexRO-LasR (SD2), pBAD-LasI and pET28a-P_{LasI}-cI-P_R-mCherry), 'OR' gate (pLexRO-P_{BAD}-ColE_{408}-mCherry (SD17) and 'N-IMPLY' gate (pLexRO-cI (SD2), pBAD-LasI and pET28a-P_{LasI}-mCherry-P_R-LasR) were cultured with differing concentration of arabinose and light intensities at 37°C for 15 h before characterization. Data represent three independent transformants.

imers, RsLOV may be used to develop various novel optogenetic tools in the future, whose light-responsive behaviours are complementary to previous optogenetic tools for bacterial and eukaryotic cells.

The eLightOn system consists of a single transcription factor, which increases its portability between cells from different species. Like many widely used repressor-operator systems, LexRO and its cognate operator can be used in other cells and are not limited to *E. coli*; an example is the use of the lac repressor–operator in a Gram-positive bacterium (37), yeast cells (38) and even mammalian cells (39–41). Owing to the modular design of LexRO, it is possible to fine tune the performance of the eLightOn system by changing each motif of LexRO, e.g. a single-residue mutation in the active site or the dimer interface of RsLOV may alter the photoadduct lifespan and induce structural changes that perturb the oligomeric state, which may affect the light sensitivity or activation kinetics of the system (10,18). Additionally, the eLightOn system can be used together with the light-off switch system (e.g. LightOff system) to simul-

taneously control the expression of two period genes that function at sequentially occurring events in living cells, providing a novel regulation behaviour to study gene regulatory networks.

Overall, we developed a light-activated gene expression system for *E. coli* showing many favorable induction characteristics, such as single-component construction, straightforward activation, highly tunable induction characteristics, precise spatiotemporal resolution and good adaptability, enabling it to be a powerful and convenient tool for the study of bacterial gene function and gene regulatory networks and for large-scale production of recombinant proteins.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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