Supplementary information, Data S1

Sequence information

Amino acid sequence of the light-switchable transcription factor LEVI

MKALTARQQEVFDLIRDHISQTGMPPTRAEIAQRLGFRSASSAEEHLKALARKGVIEIVSGASRGIRLLQEEEEGL
PLVGRVAAGEPHTLYAPGGYDIMGYLIQIMKRPNPQVELGPVDTSVALILCDLKQKDTPIVYASEAFLYMTGYSN
AEVLGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVVNFKKNGQRFVNFLTMIPVRDET
GEYRYSMGFQCETE

DNA sequence of the promoter-operator (the underlined bases represent the promoter regions; the dashed bases represent the operator regions)

*ColE promoter-LexA operato*r
TGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGTCTTGACAGGGAAAATGCAGCGGCGTAGCTTTT
ATGCTGTATATAAAACCAGTGGTTATATGTACAGTATTTATTTTAACTTATTGTTTTAAAAGTCAAAGAGGATTT
TATA

bba-J23110 promoter-LexA$_{408}$-operator
GGGGATCCTGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGTTTACGGCTAGCTCAGTCCTAGGTA
CAATGCTAGCTATGCCGTATATAAAAACGGCGTTTATATGTACGGTATTTATTTTAACTTATTGTTTTAAAAGTC
AAAGAGGATTTTATA

lacUV5-promoter-LexA$_{408}$-operator
GGGGATCCTGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGTTTACACTTTATGCTTCCGGCTCGTA
TGTTTATGCCGTATATAAAAACGGCGTTTATATGTACGGTATTTATTTTAACTTATTGTTTTAAAAGTCAAAGAG
GATTTTATA

tac-promoter-LexA$_{408}$-operator
GGGGATCCTGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGTGACAATTAATCATCGGCTCGTATAA
TGTTATGCCGTATATAAAAACGGCGTTTATATGTACGGTATTTATTTTAACTTATTGTTTTAAAAGTCAAAGAGG
ATTTTATA

T7-promoter-LexA$_{408}$-operator
GGGGATCCTGTTTTTTTGATCGTTTTCACAAAAATGGAAGTCCACAGAAATTAATACGACTCACTATAGGGCC
GTATATAAAAACGGCGTTTATATGTACGGTATTTATTTTAACTTATTGTTTTAAAAGTCAAAGAGGATTTTATA

Supplementary Methods

DNA cloning

LexA(1-87) gene fragment was amplified from the genome of *JM109(DE3)* cells, and was fused to VVD (37–186) with N56K C71V double mutations from pGAVPO[8] to generate chimeric fusion gene LexA-VVD, which was then fused to the constitutive promoter bba_J23116 from iGEM (http://parts.igem.org/Promoters/Catalog/Constitutive) and rrnB transcription terminator from pBAD/His vector using overlapping PCR and inserted into BglII and EcoRI sites in pCDFDuet1 vector (Noagen company) whose promoter and MCS region have been removed, the resulted vector was named as pLEVI. ColE promoter was synthesized by Shanghai Generay Biotech Co. Ltd., and fused to rrnB transcription terminator and mCherry gene from pU5-mCherry[8] using overlapping PCR, and then was inserted into KpnI and BglII sites in pLEVI vector, the resulted vector was named as pLEVI-mCherry. Site-directed mutagenesis, to generate sequences encoding VVD proteins with I74V mutation or M135I and M165I double mutations, to generate LexA binding domain and the cognate binding site with $LexA_{408}$ mutant, was performed according to the MutanBEST protocol (Takara). Other vectors including pLEVI-sfGFP, pLEVI-cheZ and pLEVI(408)-CcdB were obtained by substituting mCherry with genes encoding sfGFP, cheZ and CcdB-flag. cI gene fragment and R-O12 promoter were amplified from the genome of λ phage and fused to ColE promoter and mCherry gene to generate colE-cI-$P_{\lambda(R\text{-}O12)}$-mCherry DNA fragment, then was inserted into KpnI and Eco47III sites in pLEVI-mCherry to

obtain pLEVIon-mCherry vector. mCherry gene was inserted into NcoI/EcoRI of pBAD/His, BamHI/XhoI of pET28a and pDusk (Addgene: #43795) to generate pBAD/His-mCherry, pET28a-mCherry and pDusk-mCherry, respectively. ColE promoter in pLEVI(408)-mCherry was replaced by bba-J23110, lacUV5, tac and T7 promoter to generate pLEVI(408)-J23110-mCherry, pLEVI(408)-lacUV5-mCherry, pLEVI(408)-tac-mCherry and pLEVI(408)-T7-mCherry, respectively. cI was inserted into NcoI/XhoI sites of pET28a to generate pET28a-cI in which cI expression was under the control of T7 promoter. $P_{\lambda(R\text{-}O12)}$-mCherry gene cassette was amplified from pLEVIon-mCherry and cloned into pLEVI-mCherry by Acc651/EcoRI digestion., resulting in pCDF-$P_{\lambda(R\text{-}O12)}$-mCherry.

Cell growth and blue light irradiation

Unless stated otherwise, all experiments were carried out in the *E.coli* strain *JM109(DE3, ΔsulA, ΔLexA)*, whose *LexA* gene were deletion based on *JM109(DE3)* using λ red recombination system[9] (*sulA* gene should be knocked out before deletion of LexA). The cells were cultured in LB medium plus 50 $\mu g \cdot mL^{-1}$ streptomycin (streptomycin was placed by spectinomycin for *TOP10 E. coli* strain), at 260 rpm and 37℃ in DJ5-2012R Bioreactor shakers (ShiPing, Shanghai, China). For detection of light regulated gene expression, cells were illuminated by a 4.9 $W \cdot m^{-2}$ blue light emitting from an LED lamp (460 nm peak) or remained in the dark for 18 h before characterization. Neutral density filters were used to adjust the light irradiance. Light intensities were measured with a Luminometer (Sanwa, LX-2).

To test the repression kinetics of LightOff system, the starter cultures with OD_{600}~0.6 under non-inducing conditions were diluted 100-fold into fresh LB medium and transferred to dark conditions. Cells were transferred to blue light illumination at the indicated time. The aliquots were taken at indicated time points and cell growth was arrested by addition of 3.3 mg·mL^{-1} chloramphenicol and 0.4 mg·mL^{-1} tetracyclin chloramphenicol. For detecting the derepression kinetics of LightOff system in batch culture, cells were firstly cultured in blue light illumination, and then were transferred to darkness when OD_{600} reached ~0.4. To detect the derepression kinetics of LightOff system in exponential growing culture, the overnight cells diluted to OD～10^{-6} and were preconditioned for 5 h in light illumination and then were transferred to darkness. The aliquots were taken at indicated time and cell growth was stopped by antibiotic addition described above. Induction of mCherry expression from pBAD system in *TOP10* strain and pET system in *BL21(DE3)* or *BL21(DE3) pLysS* strain was carried out by adding 0.2% arabinose and 1 mM IPTG, respectively. To gauge the performance of cI/ $P_{\lambda(R\text{-}O12)}$ promoter, pET28a-cI and pCDF-$P_{\lambda(R\text{-}O12)}$-mCherry were co-transformed into *BL21(DE3) pLysS* strain, mCherry fluorescence was determined 18 h after the cells were cultured with or without 1 mM IPTG. mCherry fluorescence and OD_{600} were measured with a Synergy 2 multi-mode microplate reader (BioTek) in 96-well plates (Greiner).

To spatially control gene expression in cultured E. coli cells, *JM109(DE3, ΔsulA, ΔLexA)* cells transformed with LightOff system using mCherry as the reporter was

cultured in blue light illumination overnight. The overnight cultured cells were then mixed with LB medium with 1% agar (make sure that the temperature of the LB medium was below 50℃) and immediately poured into a Petri dish with 12-cm internal diameter and allowed to harden at room temperature for 30 min. A photomask made of 0.2 mm thick transparency was placed on the dish, and then the dish was incubated at 37 ^{o}C and illuminated from top with blue light from a blue LED array lamp of 27 cm distance. The blue light irradiation on the bacteria culture was roughly 1.1 W/m^{2}. 12 h after culture at 37 ^{o}C, image of mCherry fluorescence were acquired with 600 nm excitation and 670 nm emission filters using a Kodak In-Vivo Multispectral System FX (Carestream Health).

Quantitative RT-PCR

Total RNA was isolated from *E.coli* cells using a total RNA extraction kit (Promega) according to the manufacturer's instructions. RNA was converted to single-stranded cDNA using GoScript™ Reverse Transcription System (Promega) by the specific primers (Forward primer 5'-CCTGCCCTTCGCCTGGGACATCCTG-3' and Reverse primer 5'-TGGTCTTGACCTCAGCGTCGTAGTG-3'). For real-time quantitative reverse transcription (RT)-PCR, 2 μl of the cDNA was used for the assay with GoTaq® qPCR Master Mix (Promega) and specific primers according to the manufacturer's recommendations on a Bio-Rad CFX96 system. The specificity of amplification was verified by melt-curve analysis, and the data were collected using BioRad CFX manager software. Amplification conditions were 1 cycle of 95 ℃ for 2

min followed by 40 cycles of 95 ℃ for 15 s and 60 ℃ for 60 s, with a final melt curve analysis step (heating the PCR mixture from 65 ℃ to 95 ℃ by 0.5 ℃ every 5 s) to confirm specificity of amplification and lack of primer dimers. All samples were normalized to the 16S rRNA values and the results expressed as fold changes of cycle threshold (Ct) value relative to samples before induction by using the $2^{-\Delta\Delta Ct}$ formula.

Imaging

For live-cell fluorescence microscopy, images were acquired using an S Plan Fluor ELWD 20×, 0.45 numerical aperture (NA) objective and a digital sight camera on an Eclipse Ti inverted microscope system (Nikon), using a GFP filter for sfGFP and a Texas Red filter for mCherry. For fluorescence protein assays of native PAGE, images were acquired with 600 nm excitation and 670 nm emission filters for mCherry and 490 nm excitation and 535 nm emission filters for sfGFP using a Kodak In-Vivo Multispectral System FX (Carestream Health).

Light-controlled bacteria mobility

Light-controlled cell mobility was performed in *JM109(DE3, ΔsulA, ΔLexA, ΔcheZ)* cells, whose cheZ gene was knocked out based on *JM109(DE3, ΔsulA, ΔLexA)* using λ red recombination system. Light controlled cell mobility was conducted on semi-solid agar. To prepare semi-solid agar, 10-ml of LB medium containing 2.5 g Yeast extract, 5 g Bacto Tryptone, 5 g NaCl, 0.5% (w/v) glycerol per liter supplemented with 0.25% agar (Difco, Bacto agar) was poured into a Petri dis 7-cm

internal diameter, and allowed to harden at room temperature for 90 min. The engineered bacteria transformed with pLEVI-cheZ were picked into fresh LB and cultured at 37 ℃. When OD_{600} reached ~0.2, a suspension of cells (2-μl) was spotted onto the semi-solid agar plate and cultured in different light irradiance. All experiments were carried out at 37 ℃.

Expression of toxic protein and large scale production of recombinant protein using 5-L fomenter by LightOff system

Fermentation using LightOff system was achieved in a 5-L fermenter (Shanghai Baoxing Bioengineering Equipment Co.Ltd, China). Briefly, a single colony of *JM109(DE3, ΔsulA, ΔLexA)* transformed with pLEVI-mCherry was inoculated into LB medium with streptomycin and grown overnight. 5% (v/v) of the seed culture was inoculated to 3 L fermentation medium (2*YT). The cells were firstly grown in light irradiance at 37℃ and then transferred to darkness to induce mCherry expression, with aeration and agitation rates of 1 vvm and 500 rpm, respectively. Expression in the vector pLEVI(408)-CcdB was carried out in *JM109(DE3, ΔsulA, ΔLexA)* cells. A starter culture under blue light illumination was diluted 10-fold into fresh LB medium and transferred to dark conditions when the OD_{600} reached ~1.0 at 37℃. Cells were harvested for western blot analysis 6 h after induction.

Electrophoresis and western blot

For native gel electrophoresis, equal amounts of lysate protein were loaded onto a 15% native Tris-glycine gel and run at 20 mA for 2 h. Western blot analysis of CcdB-flag was carried out using the standard procedure. Briefly, 5 μg of the total lysate protein

were electrophoresed on 15% SDS-PAGE gel, and then transferred onto polyvinylidene fluoride (PVDF) membranes (PALL) using an electroblotter. After blocking with 0.5% casein, the membrane was probed with mouse anti-flag (1:1000; Sigma-Aldrich) antibody. Subsequently, the membrane was treated with horseradish peroxidase (HRP)-labeled secondary antibodies (1:1000; Jackson ImmunoResearch). Immunoreactivity was detected using a BM ChemiLEVIcence Blotting kit (Roche Diagnostics) according to the manufacturer's protocol on a Kodak In-Vivo Multispectral System FX (Carestream Health).

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