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Near-infrared light responsive c-di-GMP module-based AND logic gate in *Shewanella oneidensis*

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Procedure of gene circuit construction

The light responsive synthetic c-di-GMP module was composed of two genes, *bphS* and *bphO*. It was *in vitro* synthesized. In order to prevent insufficient translation rate due to rare codon, the sequence of *bphS* and *bphO* was codon optimized in *Shewanella* before synthesis. The sequence of *bphS* was obtained by reverse translation of the protein sequence of engineered BphS shown in published work.[1] And the sequence of *bphO* (RSP_4190) originating from *Rhodobacter sphaeroides* was extracted from database KEGG.[2] Codon optimization for expressing in *Shewanella* was done by JCAT,[3] and restriction enzyme sites of EcoRI, XbaI, SpeI, and PstI were avoided at the mean time. RBS-*bphS*-RBS-*bphO* was *in vitro* synthesized as a standardized biobrick. *bphS-bphO* operon contains an upstream prefix (including EcoRI and XbaI) and a downstream suffix (with SpeI and PstI). Sequentially, *bphS-bphO* operon was inserted into plasmid pYYDT.[4]

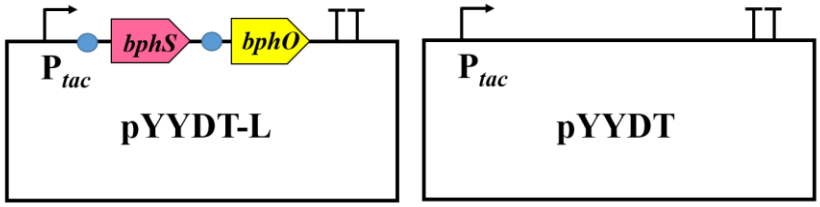

Figure S1. Schematic illustration of the gene circuit components. The gene circuit contains three key elements, IPTG inducible promoter P*tac*, diguanylate cyclase gene *bphS* and heme oxygenase gene *bphO*. IPTG inducible promoter P*tac* and double terminator (shown by **TT** in plasmid map) were included in plasmid pYYDT. The c-di-GMP module was *in vitro* synthesized as a biobrick. Blue circles represent the ribosome binding sites (RBSs). Sufficient translation of key components in the gene circuit was ensured by the strong RBS1 incorporated in front of *bphS* and *bphO*. The *bphS-bphO* operon was inserted into pYYDT, which are named as pYYDT-L.

Sequence of light responsive synthetic c-di-GMP module

An upstream prefix (including EcoRI and XbaI) and a downstream suffix (with SpeI and PstI) are heighted by green. Ribosome bind sites (RBS1)[1] are heighted by yellow. The coding sequences of gene *bphS* (2064bp) and gene *bphO* (594bp) are heighted by red and blue, respectively. The length of gene circuit is 2742bp.

GCAGAATTCGCGGCCGCTTCTAGAGaaggagatatacatATGGCTCGTGGTTGTTTAATGACTATCTCTGGTGGTACTTTCGAT
CCATCTATCTGTGAAATGGAACCAATCGCTACTCCAGGTGCTATCCAACCACACGGTGCTTTAATGACTGCTCGTGCTG
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GCTGGTGCTGCTGCTACTTTCACTGCTTTATTAGAATGGTTCACTCCATTCGTTGCTGCTCGTCGTGTTTAA T A C T A G

TA G C G G C C G C C T G C A G G

Method details for setting up the MFCs

Two-chamber MFCs separated by Nafion 117 (Gashub, Singapore) were used in experiment. The Nafion 117 membrane was pretreated as follows. First, it was boiled in 3% hydrogen peroxide for 0.5 h in water bath (80°C). After being washed with distilled water several times, it was then boiled in distilled water for 0.5 h at 80 °C. Finally, it was boiled in 0.5 M sulfuric acid for 0.5 h at 80 °C, washed, and kept in sterile distilled water before MFC setup. Carbon cloth (Gashub, Singapore) was used as the electrodes for both anode (2.5 cm × 2.5 cm, *i.e.*, the geometric area is 6.25 cm^2) and cathode (2.5 cm × 3 cm). The catholyte consists of 50 mM $K_3[Fe(CN)_6]$, 50 mM K_2HPO_4 and KH_2PO_4. The anode chamber was filled with 150 mL optimal medium. This optimal medium was M9 medium (Na_2HPO_4, 6 g /L; KH_2PO_4, 3 g/L; NaCl, 0.5 g /L; NH_4Cl, 1 g/L; $MgSO_4$, 1 mM) supplemented 5% LB and 20 mM lactate. 1 mL of overnight culture was transferred into 100 mL fresh LB medium with or without the induction of 0.5 mM IPTG, respectively. After 8h incubation (30 ℃, 200 rpm shaking), the cells were harvested and washed three times with PBS and then injected into anode chamber with a seeding optical density at 600 nm (OD_{600}) of 0.4. After inoculation, each MFC electrolyte was bubbled by nitrogen for 3 mins. A 1 kΩ external resistor was connected to both anode and cathode. MFC reactors were incubated at 30°C under four conditions (*i.e.*, no IPTG or NIR light, only IPTG (0.5 mM), only NIR light (660 nm, 7.02 mW/cm^2), and IPTG (0.5 mM) and NIR light (660 nm, 7.02 mW/cm^2)), and the voltage outputs were recorded. To further obtain MFCs performance, the polarization curves were measured by recording the voltage output at various external resistances (200k–1kΩ) when the electrical outputs of MFCs reached highest one (around 48h incubation). The voltage output was recorded after connecting the resistor when output was stable. The current density and power density were calculated by relation I=V/R·S and P=VI, respectively, where, V, R and S represents voltage (V), resistance (R) and carbon cloth area (S). From polarization curves, the maximum power densities with four input combinations were obtained.

Static biofilm assay

The plasmid with our gene circuit was transformed into wild-type *S. oneidensis* MR-1. 10 mL preculture was cultivated in LB medium (peptone, 10 g/L; yeast extract, 5 g/L; NaCl 5 g/L, pH, 7.0). After overnight growth, the culture was washed twice with phosphate buffer solution (PBS: NaCl, 8.0 g/L; $Na_2HPO_4{\cdot}12H_2O$, 3.23 g/L; $NaH_2PO_4{\cdot}2H_2O$, 0.45 g/L, pH 7.0), and re-suspended in 10 mL PBS (OD_{600} ~1.0). Subsequently, 10 μL culture was inoculated to each well of 24-well plates containing 1 mL optimal medium. 24-well plates were incubated under the induction of four input combinations: (1) no inputs; (2) only IPTG (0.5 mM); (3) only NIR light (660 nm, 7.02 mW/cm^2); and (4) IPTG (0.5 mM) and NIR light (660 nm, 7.02 mW/cm^2). After 24h incubation at 30℃, the planktonic cell was removed. 1 mL of 0.1% crystal violet was added to each well with 15 min incubation. The plates were then washed 3 times with PBS to remove unstained crystal violet. Finally, 95% ethanol was added to dissolve crystal violet in biofilm and optical density was measured at 550 nm using a microplate reader (Tecan Infinite M200 PRO). To further quantify the biovolume, the same biofilms grown on the bottom of well were imaged using confocal scanning microscopy (CLSM, Carl Zeiss Microscopy LSM 780). After removing planktonic cells, the cells were stained by 2.5 μM SYTO 9 green fluorescent nucleic acid stain. All the conditions were conducted with three biological replicates and three randomly picked images were taken for each replicate. The confocal images are shown in Fig S2. Biofilm biovolume were calculated using IMARIS (version 7.6.4, Bitplane, Zurich, Switzerland)

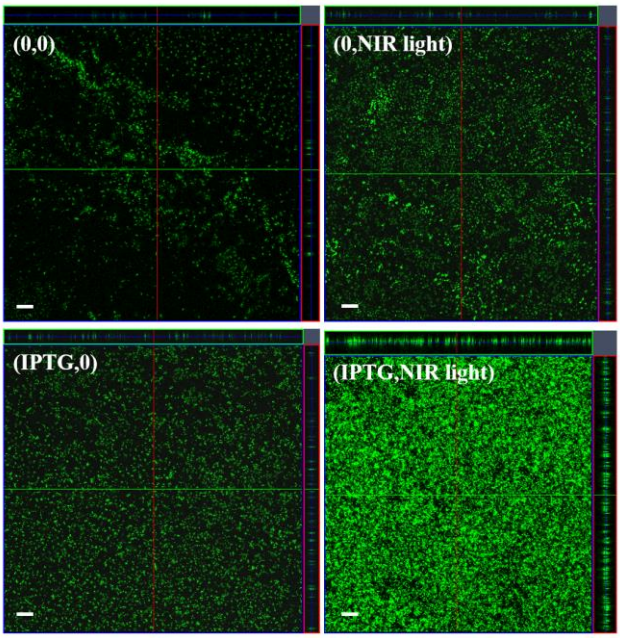

Figure S2. CLSM images of static biofilm of *S. oneidensis* harbouring plasmid pYYDT-L grown in 24-well plate under aerobic condition at 24h upon induction by four input combinations. The scale bar represents 20μm. Experiments were conducted in triplicate. For each well, three images were taken at random spot, and the representative image is shown here.

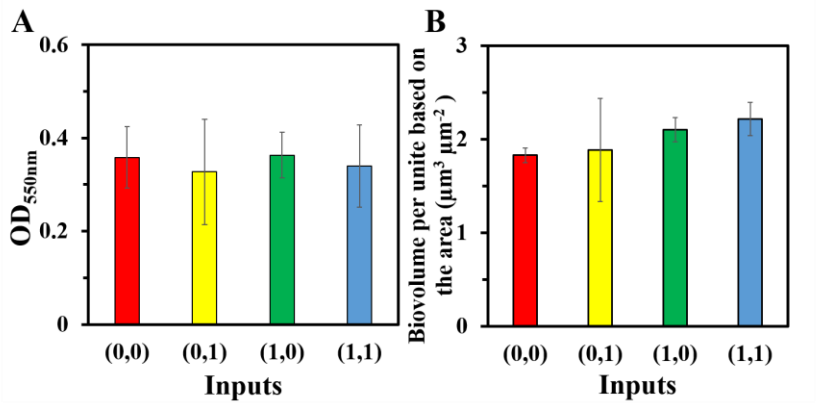

Figure S3. The performance of the vector for biofilm formation. (A) OD_{550nm} and (B) biovolume of the biofilm biomass of *S. oneidensis* harboring the vector pYYDT incubated for 24 h under four input conditions: (0, 0), no IPTG or NIR light; (1, 0), only IPTG; (0, 1), only NIR light; (1, 1), IPTG and NIR light. Experiments were conducted in triplicates.

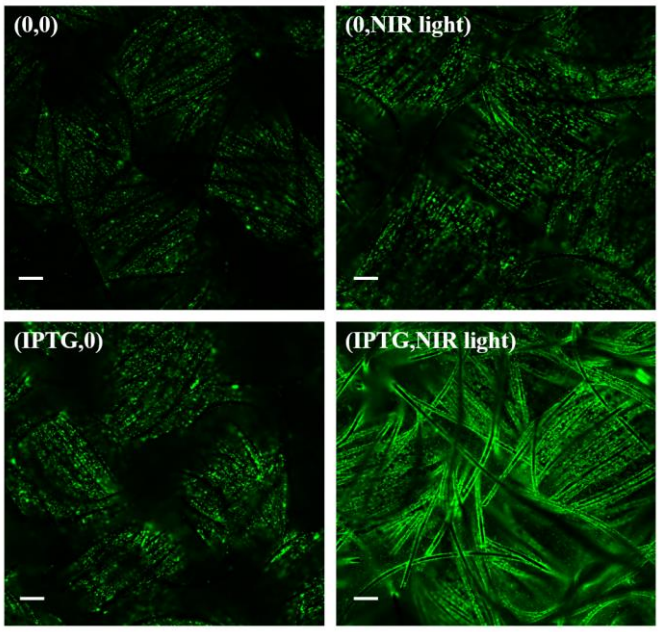

Figure S4. CLSM images of biofilms of the recombinant *S. oneidensis* containing pYYDT-L formed on the anodes of MFCs upon induction by four input combinations. MFCs experiments were conducted in triplicates for each condition. Images were taken at three random spots on each electrode, and the representative image is shown here. The scale bar represents 50μm.

References

1. M.-H. Ryu and M. Gomelsky, *ACS synthetic biology*, 2014, **3**, 802-810.
2. M. Kanehisa and S. Goto, *Nucleic acids research*, 2000, **28**, 27-30.
3. A. Grote, K. Hiller, M. Scheer, R. Münch, B. Nörtemann, D. C. Hempel and D. Jahn, *Nucleic acids research*, 2005, **33**, W526-W531.
4. Y. Yang, Y. Ding, Y. Hu, B. Cao, S. A. Rice, S. Kjelleberg and H. Song, *ACS synthetic biology*, 2015, **4**, 815-823.