A rhamnose-inducible system for precise and temporal control of gene expression in cyanobacteria

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SUPPLEMENTARY MATERIALS AND METHODS

Plasmid Construction

A list of all plasmids and oligonucleotides is provided in Table S1. Sequences of all plasmids generated in this work have been submitted to Genbank, accession numbers are MG030631 (pATM2), MG030632 (pShuttle2), MG030633 (pCK305), MG030634 (pCK306), MG030635 (pCK313), MG030636 (pCK314), MG030637 (pCK320), MG030638 (pCK321) and MG030639 (pCK324). The *E. coli-Synechocystis* sp. PCC 6803 shuttle vector pShuttle2 was constructed as follows. The region of the *Synechocystis* upstream of the *ndhB* integration site (LHA) was amplified from *Synechocystis* genomic DNA using oligonucleotides, LHA-F and LHA-R. The *kanR* gene was amplified from the *E. coli* expression plasmid pACYC177 using oligonucleotides, kan-F and kan-R. The region of the *Synechocystis* downstream of the *ndhB* integration site (RHA) was amplified from *Synechocystis* genomic DNA using oligonucleotides, RHA-F and RHA-R. These three PCR fragments were combined using overlap extension PCR. The origin of replication p15A was amplified from pACYC177 using oligonucleotides p15A-F and p15A-R and this product was combined with the 5'-phosphorylated LHA-kanR-RHA PCR fragment through blunt-end ligation. The YFP reporter shuttle vector, pATM2 was constructed

by designing and ordering a gBlock (Integrated DNA Technologies) consisting of the constitutive *Synechocystis* $P_{sll1120}$ promoter (chosen for its apparent strength on the basis of published RNAseq data[1]), a synthetic RBS (designed using the RBS calculator[2] with a predicted translation initiation rate (T.I.R.) of 12,800), and the YFP gene sequence from the iGEM Parts Registry (BBa_E0030). This fragment, along with pShuttle2 was digested with PstI and BamHI and ligated resulting in the final plasmid, pATM2.

To construct pCK306, the homology arms and p15A origin of replication of pATM2 was amplified by PCR using oligonucleotides oligoCK180 and oligoCK184, the *rhaBAD* promoter was amplified by PCR from pCK301[3] using oligonucleotides oligoCK177 and oligoCK178, the genes encoding YFP and KanR, an aminoglycoside phosphotransferase conferring resistance to kanamycin, were amplified by PCR from pATM2 using oligonucleotides oligoCK179 and oligoCK342, and the RBS and rhaS gene from E. coli strain MG1655 were amplified using oligo181 and oligo182, and these PCR products were assembled using Gibson assembly and sequence verified. To construct the Δ*rhaS* plasmid pCK305, pCK306 was amplified by PCR using oligonucleotides oligoCK184 and oligoCK342 and the product phosphorylated and ligated using the NEB Site-directed mutagenesis kit. The CRP-binding site deletion plasmids, pCK313 and pCK314 were constructed by amplifying pCk305 or pCK306 respectively with oligonucleotides oligoCK180 and oligoCK332 and the product phosphorylated and ligated using NEB Site-directed mutagenesis kit. The *rhaS* RBS variants pCK320 and pCK321 were constructed by amplifying pCK306 with oligonucleotides oligoCK340 and oligoCK342 (pCK320) or oligoCK341 and oligoCK342 (pCK321) and the product phosphorylated and ligated using NEB Site-directed mutagenesis kit. Finally the *rhaBAD* promoter deletion plasmid, pCK324 was constructed by amplifying pCK321 with oligonucleotides oligoCK179 and oligoCK180 and the product phosphorylated and ligated using NEB Site-directed mutagenesis kit.

Name	**Details**
pShuttle2	Medium copy plasmid (p15A), with 2054 nucleotides of homology to the *Synechocystis* sp. PCC 6803 chromosome, allowing integration of DNA after the first 34 nucleotides of ssl0410 (adjacent to *ndhB*), *kanR* gene encoding KanR, an aminoglycoside phosphotransferase
pATM2	As pShuttle2 but containing the sll1120 promoter of *Synechocystis* sp. PCC 6803, a synthetic RBS and eYFP.

pCK305	As pATM2, but with the *sl1120* promoter replaced with the *E. coli rhaBAD* promoter
pCK306	As pCK305, but with the *E. coli rhaS* RBS and gene inserted downstream of the *kanR* gene
pCK313	As pCK305, but with the catabolite repression protein (CRP) - binding sites in the *rhaBAD* promoter deleted
pCK314	As pCK306, but with the catabolite repression protein (CRP) - binding sites in the *rhaBAD* promoter deleted
pCK320	As pCK306, but with a synthetic RBS with a translation initiation rate (TIR: 5,000) replacing the native *E. coli rhaS* RBS.
pCK321	As pCK306, but with a synthetic RBS with a translation initiation rate (TIR: 18,000) replacing the native *E. coli rhaS* RBS.
pCK324	As pCK321, but with the *rhaBAD* promoter deleted
oligoCK178	atttctacctcctttgtatattataaacttacccgattcattacgaccagtctaaaaagc
oligoCK179	tgaatcgggtaagtttataatatacaaaggaggtagaaatggtgagcaagggcgaggagc
oligoCK180	ctgcagtctcctttaaatcggtttctatagttacactcattggcttttgcctgcaaagc
oligoCK181	taaattgcagtttcatttgatgctcgatgagtttttctaatatttcgccgtgttgacgac
oligoCK182	ttttggaggtcatcgagtttggtcttaatttcccccaagattattgcagaaagccatccc
oligoCK183	tcttgggggaaattaagacc
oligoCK332	ttcatctttccctggttgccaatgg
oligoCK333	atttgctgaattgtggaagggcg
oligoCK340	tgcccctatatttcaccttcgagattacccgttatgaccgtattacatagtgtgg
oligoCK341	gagcctttcgaagcagcaatcaaatgaccgtattacatagtgtgg
oligoCK342	ttagaaaaactcatcgagcatcaaatgaaac
LHA-F	5’-Phos-gtgggtcaatccctcggtttg
LHA-R	5’-Phos-ggatcctggagcataactgcagtctcctttaaatcggtttctatag
kan-F	ctgcagttatgctccaggatccaaagccacgttgtgtctcaaaatc

kan-R	taatttcccccaagattagaaaaactcatcgagcatcaaatg
RHA-F	gatgagtttttctaatcttgggggaaattaagaccaaac
RHA-R	5'-Phos-gttctatttgcccgtattctgcc
p15A-F	aaatctggagccggtgagcgt
p15A-R	cgcatcttcccgacaacgcag

Table S1. Plasmids and oligonucleotides used in this study. 5'-phosphorylated primers denoted 5'-Phos.

Figure S1. Sequence alignment of RNA polymerase sigma 70 factors from *E. coli* and *Synechocystis* sp. PCC 6803. RpoD (NP_417539.1) of the *E. coli* K12 strain MG1655 was

aligned pairwise with SigA (ALJ69094.1) of *Synechocystis* sp. PCC 6803 using EMBOSS Needle[4] accessed at https://www.ebi.ac.uk/Tools/psa/. Box 1. Residues involved in binding to the -10 promoter region. Box 2. Residues involved in binding to the -35 promoter region. Underlined are the two residues in the *E. coli* sigma 70 factor, K593 and R599, required for interaction with two residues of RhaS (D250 and D241 respectively) and the conserved residues found in the *Synechocystis* ortholog (R412 and K406 respectively).

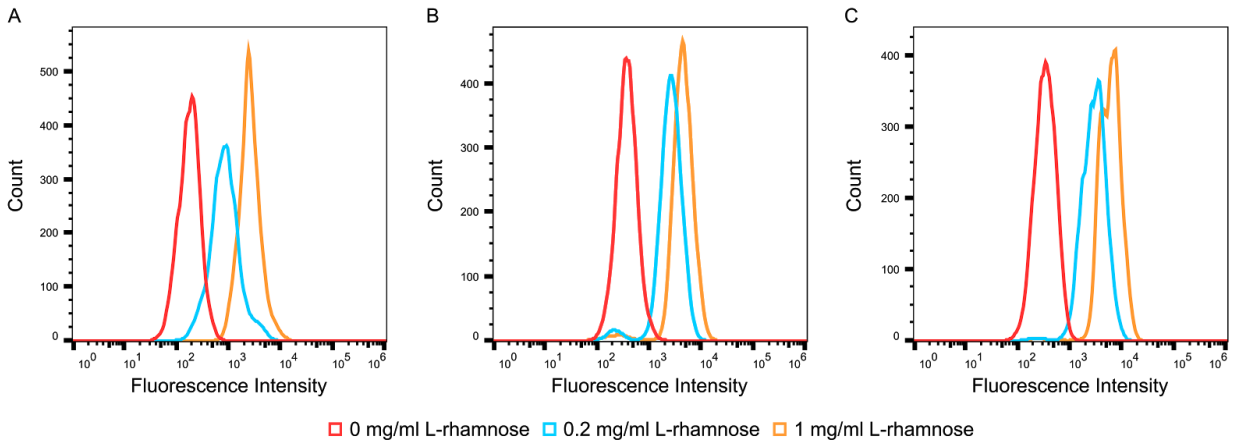

Figure S2. Distribution of expression levels from the *rhaBAD* promoter in populations of *Synechocystis* sp. PCC 6803 cells grown photoautotrophically or mixotrophically. A. *Synechocystis* cells containing pCK306 (P_{rhaBAD}, YFP, +*rhaS*) grown in photoautotrophic conditions, fluorescence intensity of 10,000 cells assayed by flow cytometry after 120 h. B. Same strain grown in mixotrophic conditions (supplemented with 5 mM D-glucose), fluorescence intensity assayed by flow cytometry after 120 h. C. Same strain grown in mixotrophic conditions (supplemented with 5 mM D-glucose), fluorescence intensity assayed by flow cytometry after 215 h.

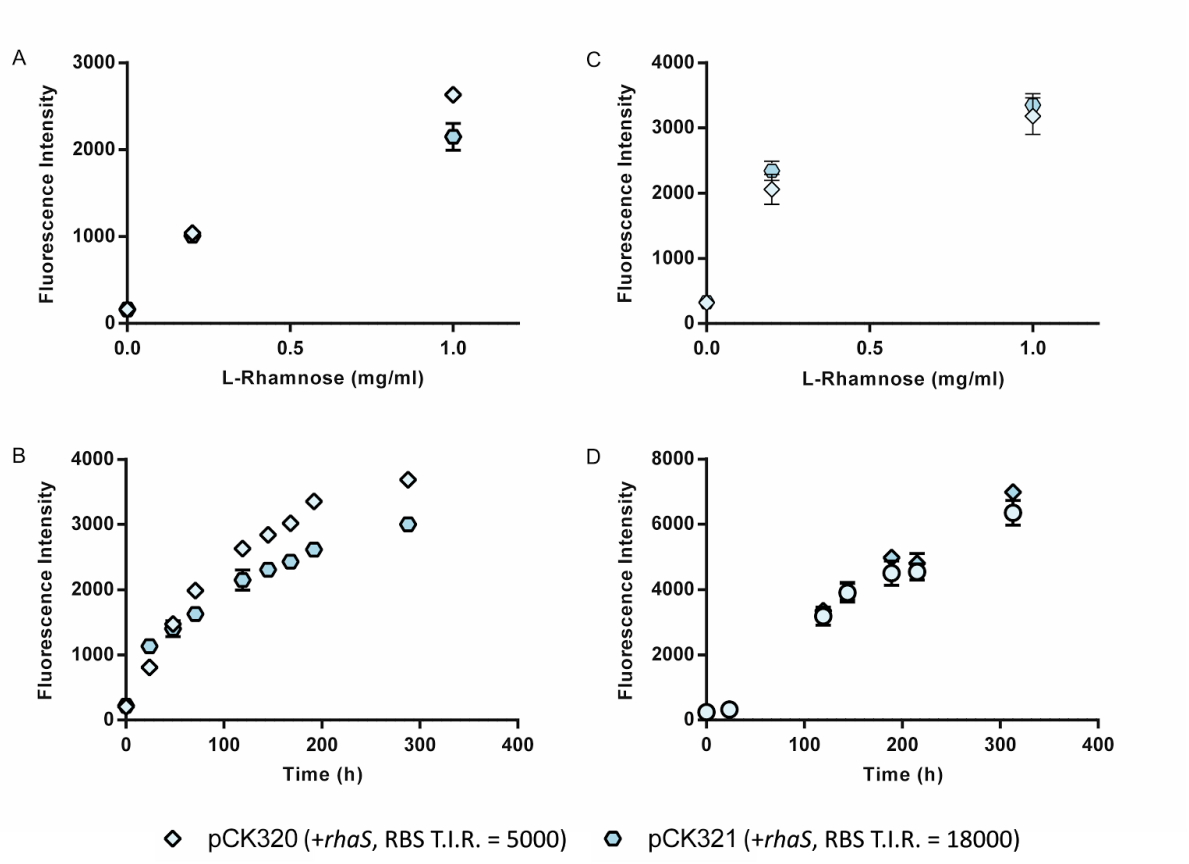

Figure S3. Response to concentration of L-rhamnose inducer and timecourse of induction of the *rhaBAD* promoter when the strength of the *rhaS* RBS has been increased. A. *Synechocystis* sp. PCC 6803 cells containing either pCK320 (*rhaBAD* promoter, YFP, *rhaS*, rhaS RBS T.I.R. of 5,000) or pCK321 (*rhaBAD* promoter, YFP, *rhaS*, rhaS RBS T.I.R. of 18,000) were cultured in BG11 media supplemented with specified concentrations of L-rhamnose in photoautotrophic conditions and the fluorescence intensity of 10,000 cells measured after 116 h using flow cytometry. B. The same strains of *Synechocystis* were cultured in BG11 media supplemented with L-rhamnose to a final concentration of 1 mg/ml in photoautotrophic conditions and the fluorescence intensity of 10,000 cells measured at specified timepoints using flow cytometry. C. Equivalent experiment to A but strains cultured in BG11 supplemented with 5 mM D-glucose (mixotrophic growth). D. Equivalent experiment to B but strains cultured in BG11 supplemented with 5 mM D-glucose (mixotrophic). Error bars shown are the standard deviation of the mean for three independent biological replicates.

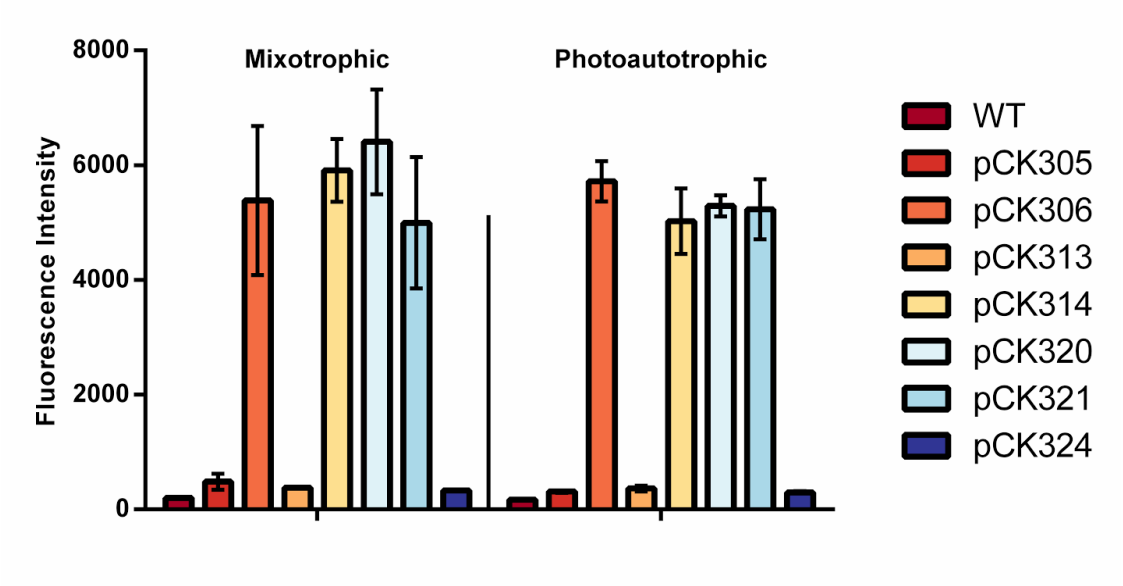

Figure S4. Comparison of response of *Synechocystis* cells containing different reporter plasmids with 1 mg/ml L-rhamnose in both photoautotrophic and mixotrophic growth conditions. Wild-type (WT) *Synechocystis* sp. PCC 6803 cells or strains containing either pCK305 (*rhaBAD* promoter YFP, no *rhaS*), pCK306 (*rhaBAD* promoter, YFP, *rhaS*), pCK313 (*rhaBAD* promoter YFP, no *rhaS*, CRP-binding site deleted), pCK314 (*rhaBAD* promoter, YFP, *rhaS*, CRP-binding site deleted), pCK320 (*rhaBAD* promoter, YFP, *rhaS*, rhaS RBS with T.I.R. of 5,000), pCK321 (*rhaBAD* promoter, YFP, *rhaS,* rhaS RBS with T.I.R. of 18,000) or pCK324 (no *rhaBAD* promoter, YFP, *rhaS*, rhaS RBS with T.I.R. of 18,000) were cultured in BG11 media supplemented with 1 mg/ml of L-rhamnose in photoautotrophic (no glucose) or mixotrophic (supplemented with 5 mM D-glucose) and the fluorescence intensity of 10,000 cells measured after 191 h using flow cytometry. Error bars shown are the standard deviation of the mean for three independent biological replicates.

SUPPLEMENTARY REFERENCES

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