Comparative dose-response analysis of inducible promoters in cyanobacteria.

Anna Behle[1], Pia Saake[1], Anna T. Germann[1], Dennis Dienst[2], Ilka M. Axmann[1]*

[1]Institute for Synthetic Microbiology, Heinrich Heine University Duesseldorf, Duesseldorf, Germany

[2]Department of Chemistry – Ångström, Uppsala University, Uppsala, Sweden

*E-Mail: Ilka.Axmann@hhu.de | Website: http://www.synmikrobiologie.hhu.de

Supporting Information

Supplementary File 1: BMG ClarioStar protocol for measurement of mVenus fluorescence in *Synechocystis* sp. PCC 6803

Supplementary File 2: Supporting Tables and Figures for this work.

Table S1: Detailed descriptions and sequences of all relevant genetic modules used in this work

Fig. S1: 5'RACE results from TSS-mapping of P_{coaT}.

Fig. S2: Growth of control strain and P_{coaT}:mVenus strain in different Co^{2+} concentrations.

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5'RACE of the Co^{2+}-inducible promoter P_{coaT}

To map the transcription start site (TSS) of P_{coaT}, we performed 5'RACE, as described in detail on protocols.io (dx.doi.org/10.17504/protocols.io.jk7ckzn). Five putative TSS were discovered, three of which occurred at a higher frequency (Fig. S1 A).

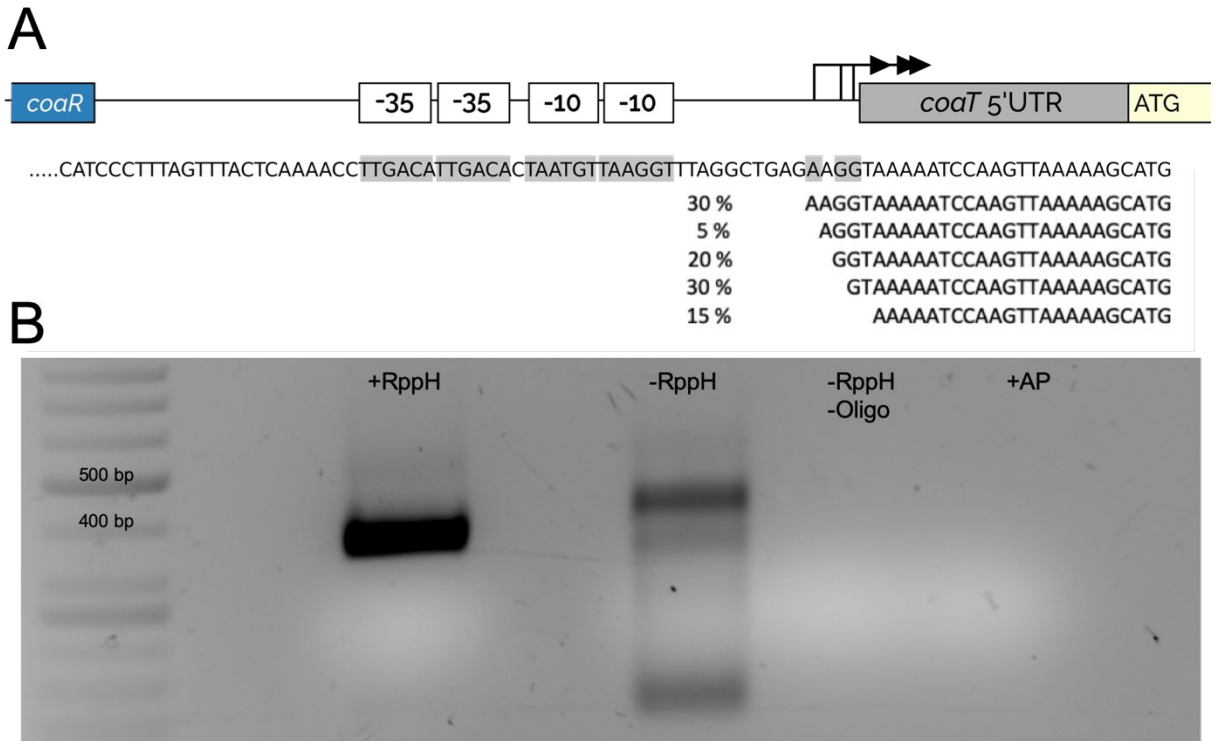


Fig. S1: 5'RACE results from TSS-mapping of P_{coaT}.
A: Top: Schematic overview of genetic construct used. Putative -10, -35 and +1 are highlighted in grey. Bottom: Putative transcription start sites based on sequencing results from 5'RACE. The percentages indicate the frequency of each individual start site as determined by sequencing. A total of 20 clones were sequenced.
B: 1% agarose gel electrophoresis of PCRs on *coaT*-specific *Synechocystis* cDNA using adapter-specific and gene-specific, nested primer. +RppH: RNA was treated with RppH (pyrophosphohydrolase), ligated to RNA-linker, then, cDNA synthesis was performed with gene specific primer. -RppH: untreated RNA was ligated to RNA-linker, then, cDNA synthesis was performed with gene specific primer. -RppH -Oligo: cDNA from untreated RNA control without ligated RNA-linker. +AP: RNA was treated with Alkaline Phosphatase, then ligated to RNA-linker, then, cDNA synthesis was performed with gene specific primer.

Increased Co^{2+} toxicity in the presence of an additional plasmid-encoded *coaR* copy

Fig. S2 shows the growth behavior of the two different *Synechocystis* strains used for the dose-response assay under different Co^{2+} concentrations. While the control strain containing the unmodified pSHDY plasmid shows decreased growth at 30 µM $CoCl_2$ (Fig. S2 A), the strain encoding an additional *coaR* copy (pSHDY P_{coaT}:mVenus) already shows severe growth defects at 10 µM $CoCl_2$ (Fig. S2 B).

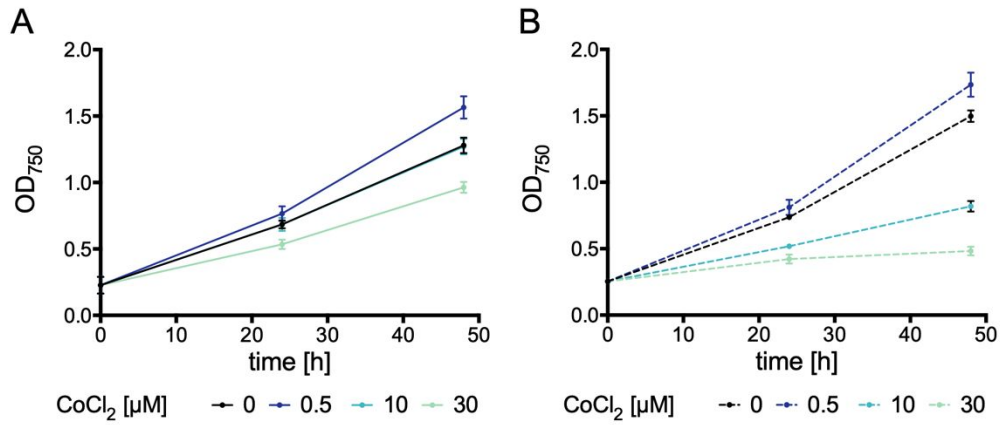


Fig. S2: Growth of control strain and P_{coaT}:mVenus strain in different Co^{2+} concentrations.
A: Cobalt-dependent growth behavior of *Synechocystis* with unmodified pSHDY over time.
B: Cobalt-dependent growth behavior of *Synechocystis* with pSHDY P_{coaT}:mVenus over time.
Optical density was measured at 750 nm.

Evaluation of chemical crosstalk among single promoter constructs

In order to investigate inducer specificity, each single promoter construct was also induced with all possible combinations of inducers.

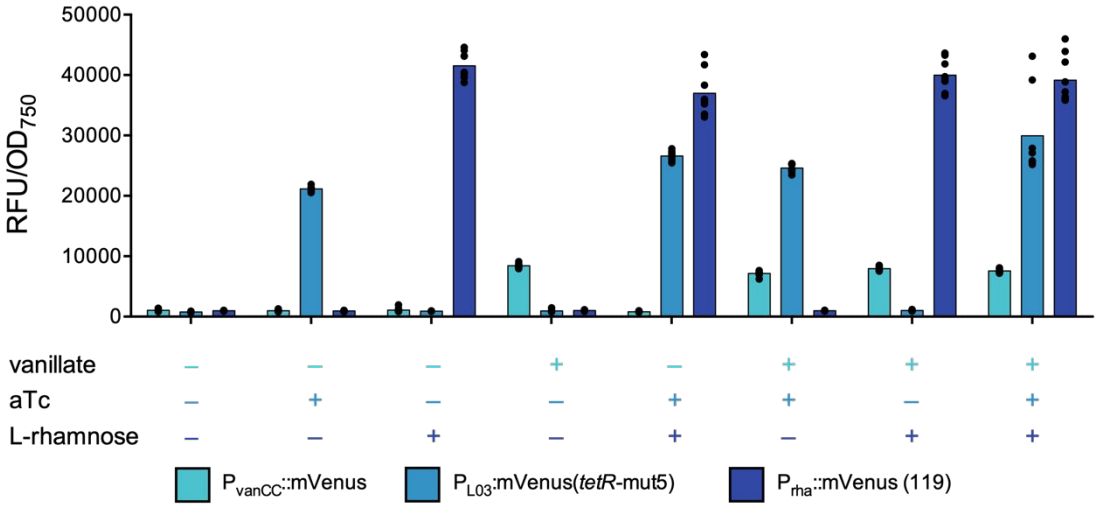
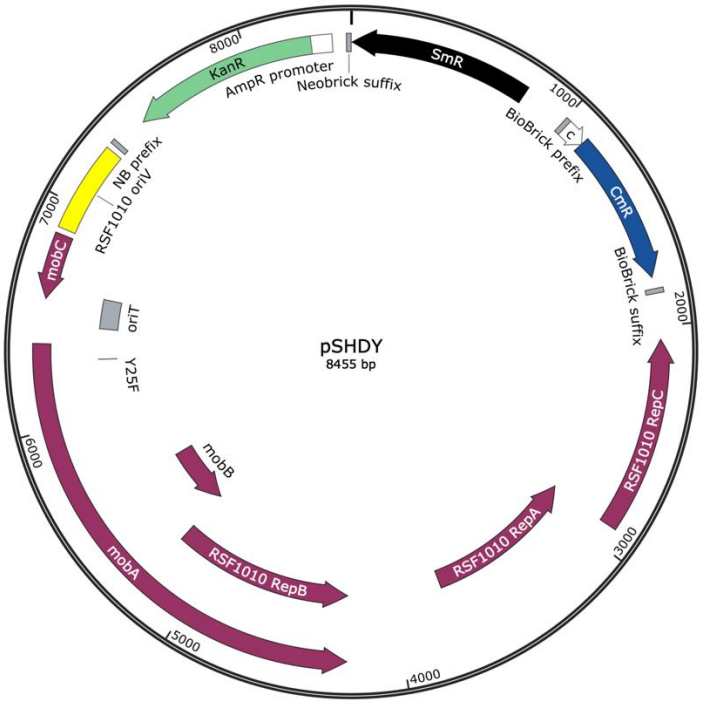


Fig. S3: Evaluation of chemical crosstalk between different promoter constructs and inducers.
Each separate promoter construct was induced with the different chemical inducers or combinations thereof.
Three biological replicates each were cultured in BG11 + inducer (10 mM rhamnose, 1 mM vanillate or 1 µM aTc or combinations thereof, marked by a + when present or a – when absent) and fluorescence and OD_{750} were measured in a microplate reader after 24 h.

A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SmR
KanR
AmpR promoter
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BioBrick prefix
CmR
BioBrick suffix
NB prefix
RSF1010 oriV
mobC
oriT
Y25F
mobB
mobA
RSF1010 RepB
RSF1010 RepA
RSF1010 RepC
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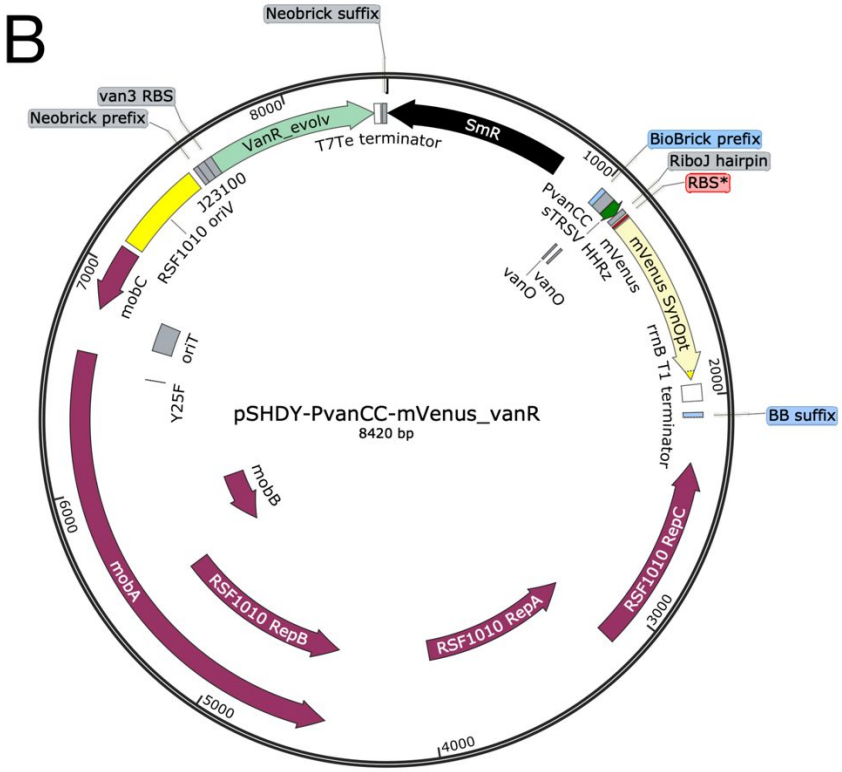
4000
5000
6000
7000
8000

B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van3 RBS
Neobrick prefix
VanR_evolv
T7Te terminator
SmR
BioBrick prefix
RiboJ hairpin
RBS*
J23100
RSF1010 oriV
PvanCC
sTRSV
HHRz
mVenus
mVenus SynOpt
vanO
vanO
rrnB T1 terminator
BB suffix
mobC
oriT
Y25F
mobB
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RSF1010 RepA
RSF1010 RepC
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5000
6000
7000
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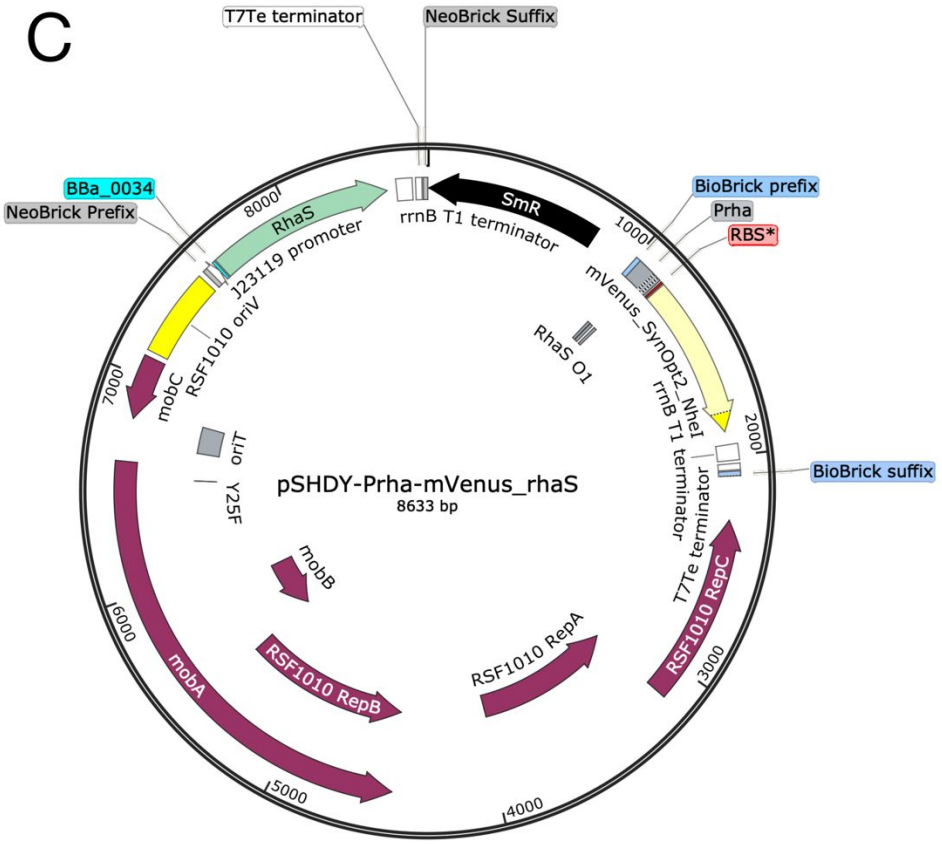
C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NeoBrick Suffix
BBa_0034
NeoBrick Prefix
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RhaS
J23119 promoter
SmR
rrnB T1 terminator
1000
BioBrick prefix
Prha
RBS*
mVenus_SynOpt2_NheI
RhaS O1
RSF1010 oriV
7000
mobC
rrnB T1 terminator
2000
BioBrick suffix
oriT
Y25F
pSHDY-Prha-mVenus_rhaS
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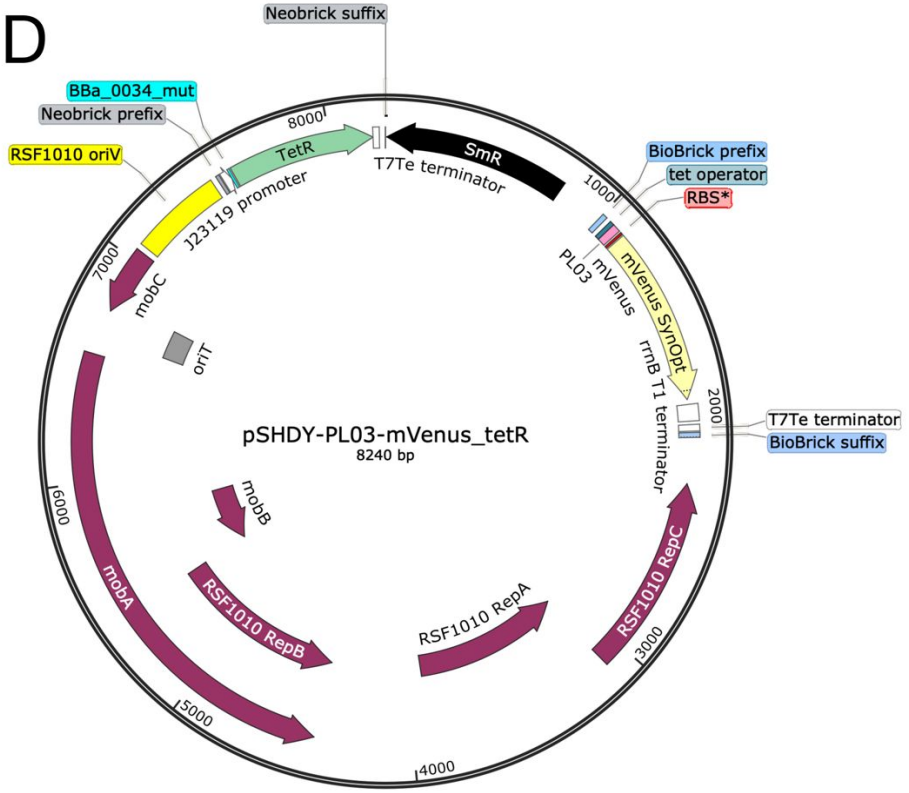
4000

D


Neobrick suffix
BBa_0034_mut
Neobrick prefix
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TetR
SmR
T7Te terminator
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J23119 promoter
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tet operator
RBS*
PL03
mVenus
mVenus SynOpt
7000
mobC
rrnB T1 terminator
2000
T7Te terminator
BioBrick suffix
oriT
pSHDY-PL03-mVenus_tetR
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RSF1010 RepA
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3000
5000
4000

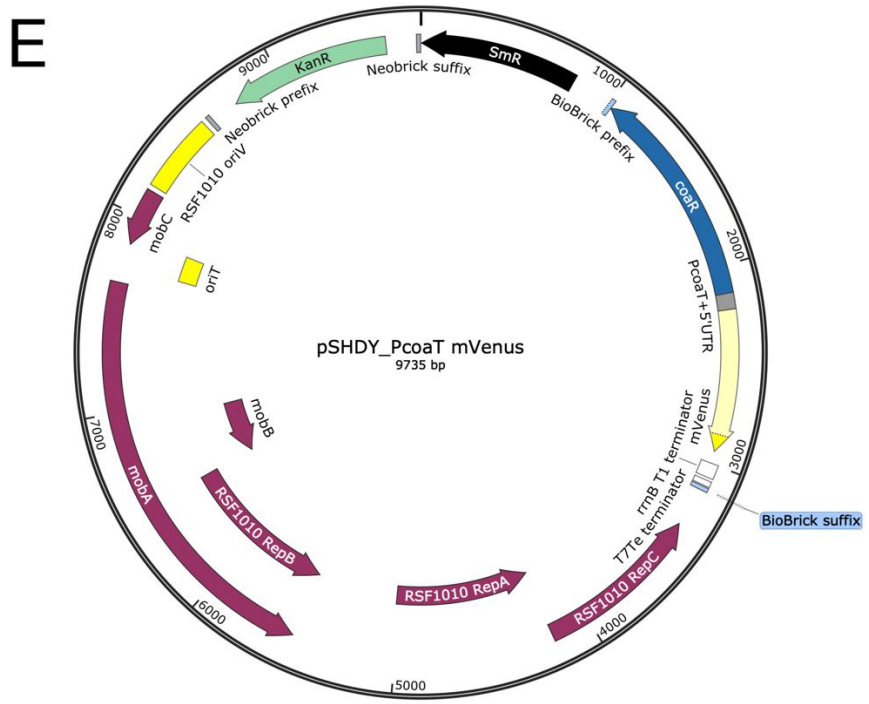

Fig. S4: Maps of vectors used.
A: pSHDY **B:** pSHDY_P_{vanCC}:mVenus-P_{J23100}:vanR **C:** pSHDY_P_{rha}:mVenus-P_{J23119}:rhaS **D:** pSHDY_P_{L03}:mVenus-P_{J23119}:tetR-mut5 **E:** pSHDY_P_{coaT}:mVenus. Plasmid maps were created using SnapGene.

Changes of the characteristic vanillate absorption spectra over time

Since vanillate has a characteristic absorption spectrum[1], we investigated whether this changed over time. We therefore conducted culturing experiments in our incubators, supplementing either BG11 medium only, or *Synechocystis* culture carrying the empty vector only with either 0.1 mM vanillate solved in 100 % ethanol, or an equivalent volume of 100 % ethanol. Indeed, our preliminary results show that the absorption spectrum of vanillate in the supernatant of the *Synechocystis* culture changes, with the two absorption peaks shifting towards 300 nm (Fig. S5). The temporal behavior of this shift is consistent with the loss of signal observed in the fluorescence experiments. In contrast, this does not occur in BG11 only, suggesting chemical stability both in light and over time.

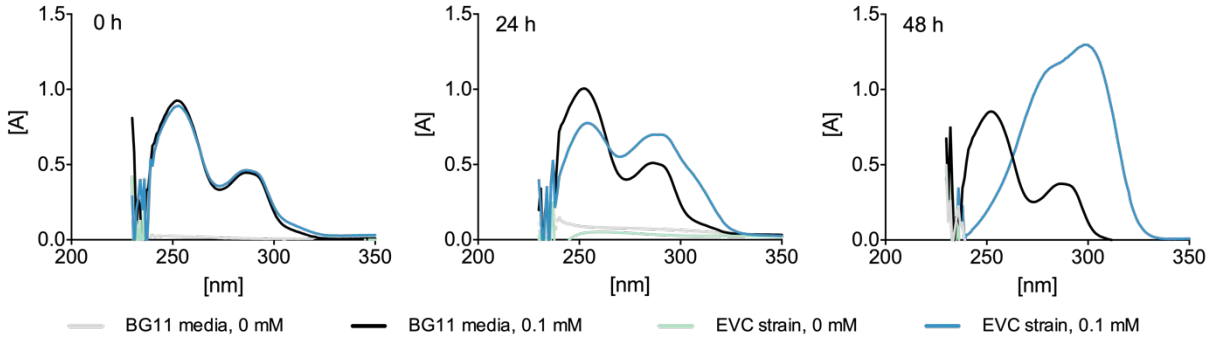

Fig. S5: Absorption spectra of culture supernatants supplemented with different concentrations of vanillate.

BG11 media (grey, black) or *Synechocystis* carrying the empty vector was supplemented with 0.1 mM vanillate or an equivalent volume of 100 % ethanol and incubated over time. 1 mL culture volume was sampled and centrifuged, and the absorption spectra of the supernatants were measured 0 h (left), 24 h (middle) and 48 h (right) post-induction, using UV-cuvettes. No absorbance was detected in any sample at wavelengths above 350 nm.

Name	Part type	Sequence	Origin	Notes
P_{vanCC}::riboJ	Promoter + insulator	gattggatccaattgacagctagctcagtcctaggtaccattggatccaatagctgtcaccggatgtgctttccggtctgatgagtccgtgaggacgaaacagcctctacaaataattttgtttaa	[2]	Amplified from addgene plasmid pAJM.714[2]
RBS*	RBS	tactagagtagtggaggttactag	[3]	
mVenus_ASV	CDS	atggctagcaaaggagaggaactgtttaccggtgtggtacccattttagtagaattggatggtgatgtgaacggccacaagttcagcgtttccggggaaggcgaaggggatgcaacctatgg aaagctaactttgaaactcatttgcactaccggtaaactccccgtcccctggcctacgttagtcacgaccctcggttatggtctgcaatgttttgcccgttatcccgatcatatgaaacaacacgattt ctttaaatctgccatgccagagggatatgttcaagaacggacaattttctttaaggatgatggtaattataaaacccgtgcagaagtcaaatttgaaggtgatacactcgtgaatcgcatcgaact aaaaggtattgactttaaggaggatggaaacattctcggccataagttagaatacaattacaacagccataatgtgtacattactgcggataaacaaaaaaacggtatcaaagccaactttaa aattcgacataacatcgaagatggtggcgttcagttagcggatcactaccaacaaaatactcccattggagacggaccagtcctgctgcctgataaccattatctgtcttatcaatccaaactgtc caaggatccgaatgaaaagcgggatcacatggttctgttggagtttgtgaccgcggcaggtattactctcggaatggacgaactatacaaaaggcctgccgccaacgacgaaaactacgct gcatcagtttaa	This work	Codon-optimized for *Synechocystis*, with C-terminal ASV degradation tag (highlighted in grey).
P_{rha}	Promoter	gccacaattcagcaaattgtgaacatcatcacgttcatctttccctggttgccaatggcccattttcctgtcagtaacgagaaggtcgcgaattcaggcgctttttagactggtcgtaatgaa	[4]	
P_{L03}	Promoter	gtacgcaattggcatgcgcattccctatcagtgatagagattgacatccctatcagtgatagatataatggccact	[5]	
P_{coaT}	Promoter +5'UTR	ccctttagtttactcaaaaccttgacattgacactaatgttaaggtttaggctgagaaggtaaaaatccaagttaaaaagc	*Synechocystis*	81 bp upstream of the *coaT* gene
RBSvan3::*vanR*	RBS + CDS	gcttaaactaacgaacgtaaataaggaggatagacatggacatgcctcgtattaaaccgggtcagcgtgttatgatggcactgcgtaaaatgattgcaagcggtgaaatcaaaagtggtgaa cgtattgcagaaattccgaccgcagcagcactgggtgttagccgtatgccggttcgtatcgcactgcgttcactggaacaagaaggtctggttgttcgtctgggtgcacgtggttatgcagcccgt ggtgttagcagcgatcagattcgtgatgcaattgaagttcgtggtgttctggaaggttttgcagcacgtcgtctggcagaacgtggtatgaccgcagaaacccatgcacgttttgttgtactgattgc agaaggtgaagcactgtttgcagcccggtcgcctgaatggtgaagatctggatcgttatgccgcatataatcaggcatttcatgatacctggttagcgcagcaggtaatggtgcagttgaaagc gcactggcacgtaatggttttgaaccgtttgcagcagccggtgcactggccctggatctgatggacctgtctgccgaatatgaacatctgctggcagcacatcgtcagcatcaggcagttctgga tgcagttagctgtggtgatgccgaaggtgcagaacgtattatgcgtgatcatgcactggcagcaattcgtaatgcaaaagtttttgaagcagcagcaagcgcaggcgcaccgctgggtgcag catggtcaattcgtgcagattga	[2]	Amplified from addgene strain sAJM.1504[2]
P_{J23119}	Promoter	ttgacagctagctcagtcctaggtataatgctagc	iGEM registry	Bba_J23119
P_{J23111}	Promoter	ttgacggctagctcagtcctaggtatagtgctagc	iGEM registry	Bba_J23119
P_{J23100}	Promoter	ttgacggctagctcagtcctaggtacagtgctagc	iGEM registry	Bba_J23119
Bba_0034	RBS	aaagaggagaaatactag	iGEM registry	Bba_B0034
rhaS	CDS	atgaccgtattacatagtgtggatttttttccgtctggtaacgcgtccgtggcgatagaaccccggctcccgcaggcggattttcctgaacatcatcatgattttcatgaaattgtgattgtcgaacatg gcacgggtattcatgtgtttaatgggcagccctataccatcaccggtggcacggtctgtttcgtacgcgatcatgatcggcatctgtatgaacataccgataatctgtgtctgaccaatgtgctgtat cgctcgccggatcgatttcagtttctcgccgggctgaatcagttgctgccacaagagctggatgggcagtatccgtctcactggcgcgttaaccacagcgtattgcagcaggtgcgacagctgg ttgcacagatggaacagcaggaaggggaaaatgatttaccctcgaccgccagtcgcgagatcttgtttatgcaattactgctcttgctgcgtaaaagcagtttgcaggagaacctggaaaaca gcgcatcacgtctcaacttgcttctggcctggctggaggaccattttgccgatgaggtgaattgggatgccgtggcggatcaattttctctttcactgcgtacgctacatcggcagcttaagcagca aacgggactgacgcctcagcTatacctgaaccgcctgcgactgatgaaagcccgacatctgctacgccacagcgaggccagcgttactgacatcgcctatcgctgtggattcagcgacagt aaccacttttcgacgctttttcgccgagagtttaactggtcaccgcgtgatattcgccagggacgggatggctttctgcaataa	*E. coli*	Amplified from *E. coli* genome. Contains an Arg218Leu mutation.
P_{J23119}-mut5	Promoter + RBS	ttgacagctagctcagtcctaggtataatgctagcaagagagaaatactag	this work	
P_{J23119}-mut5	Promoter + RBS	ttaacagctagctcagtcctaggtataatgctagcaaagaggagaaatactag	this work	
tetR	CDS	atgtccagattagataaaagtaaagtgattaacagcgcattagagctgcttaatgaggtcggaatcgaaggtttaacaacccgtaaactcgcccagaagctaggtgtagagcagcctacattg tattggcatgtaaaaaataagcgggctttgctcgacgccttagccattgagatgttagataggcaccatactcacttttgccctttagaaggggaaagctggcaagatttttacgtaataacgcta aaagttttagatgtgctttactaagtcatcgcgatggagcaaaagtacatttaggtacacggcctacagaaaaacagtatgaaactctcgaaaatcaattagccttttttatgccaacaaggtttttc actagagaatgcattatatgcactcagcgctgtggggcattttactttaggttgcgtattggaagatcaagagcatcaagtcgctaaagaagaaagggaaacacctactactgatagtatgccg ccattattacgacaagctatcgaattatttgatcaccaaggtgcagagccagccttcttattcggccttgaattgatcatatgcggattagaaaaacaacttaaatgtgaaagtgggtcctaa	iGEM registry	Bba_P0440. *tetR* was amplified omitting the LVA degradation tag.
P_{rnpB}	Promoter	ttcaatgcggtccaatacctcccctgcccaactgggtaagctcgcggctccactgagtaatacagacaaggctaaacaggcaaatttttcattggtcaactcctagcaccaatttcccaagact acggagggggcaatgaagtttcaattaattggggtcacaaaccacagcggcctatggctctaatcaatggcacactagaaaaa	*Synechocystis*	Amplified from *Synechocystis* genome
P_{rbcL}	Promoter + 5'UTR	cagtcaatggagagcattgccataagtaaaggcatcccctgcgtgataagattacctcagaaaacagatagttgctgggttatcgcagatttttctcgcaaccaaataactgtaaataataact gtctctggggcgacggtaggctttatattgccaaatttcgcccgtgggagaaagctaggctatt	*Synechocystis*	Amplified from *Synechocystis* genome
P_{cpc560}	Promoter + 5'UTR	cacctgtagagaagagtccctgaatatcaaaatggtgggataaaaagctcaaaaaggaaagtaggctgtggttccctaggcaacagtcttccctaccccactggaaactaaaaaaacgag aaaagttcgcaccgaacatcaattgcataattttagccctaaaacataagctgaacgaaactggttgtcttcccttcccaatccaggacaatctgagaatcccctgcaacattacttaacaaaaa agcaggaataaaattaacaagatgtaacagacataagtcccatcaccgttgtataaagttaactgtgggattgcaaaagcattcaagcctaggcgctgagctgtttgagcatcccggtggccc ttgtcgctgcctccgtgtttctccctggatttatttaggtaatatctctcataaatccccgggtagttaacgaaagttaatggagatcagtaacaataactctagggtcattactttggactccctcagttt atccgggggaattgtgtttaagaaaatcccaactcataaagtcaagtaggagattaattca	*Synechocystis* [6]	Amplified from *Synechocystis* genome

Table S1: Detailed descriptions and sequences of all relevant genetic modules used in this work.

Supplementary References

(1) Robbins, R. J. (2003) Phenolic acids in foods: An overview of analytical methodology. *J. Agric. Food Chem. 51*, 2866–2887.
(2) Meyer, A. J., Segall-Shapiro, T. H., Glassey, E., Zhang, J., and Voigt, C. A. (2019) *Escherichia coli* “Marionette” strains with 12 highly optimized small-molecule sensors. *Nat Chem. Biol. 15*, 196–204.
(3) Heidorn, T., Camsund, D., Huang, H.-H., Lindberg, P., Oliveira, P., Stensjö, K., and Lindblad, P. (2011) Synthetic biology in cyanobacteria engineering and analyzing novel functions. *Methods Enzymol. 497*, 539–579.
(4) Kelly, C. L., Taylor, G. M., Hitchcock, A., Torres-Méndez, A., and Heap, J. T. (2018) A Rhamnose-Inducible System for Precise and Temporal Control of Gene Expression in Cyanobacteria. *ACS Synth. Biol. 7*, 1056–1066.
(5) Huang, H.-H., and Lindblad, P. (2013) Wide-dynamic-range promoters engineered for cyanobacteria. *J. Biol. Eng. 7*, 1–11.
(6) Zhou, J., Zhang, H., Meng, H., Zhu, Y., Bao, G., Zhang, Y., Li, Y., and Ma, Y. (2014) Discovery of a super-strong promoter enables efficient production of heterologous proteins in cyanobacteria. *Sci. Rep. 4*, 235–6.