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Item Type	Article
Authors	Romano, Edoardo;Baumschlager, Armin;Akmeriç, Emir Bora;Palanisamy, Navaneethan;Houmani, Moustafa;Schmidt, Gregor;Öztürk, Mehmet Ali;Ernst, Leonard;Khammash, Mustafa;Di Ventura, Barbara
Citation	Romano, E., Baumschlager, A., Akmeric, E. B., Palanisamy, N., Houmani, M., Schmidt, G., Öztürk, M. A., Ernst, L., Khammash, M., & Di Ventura, B. (2021). Engineering AraC to make it responsive to light instead of arabinose. <i>Nature chemical biology</i> , 17(7), 817-827.
DOI	10.1038/s41589-021-00787-6
Publisher	Springer Nature
Journal	Nature Chemical Biology
Download date	2026-05-19 16:30:07
Link to Item	http://hdl.handle.net/10034/629466

1 **Engineering AraC to make it responsive to light instead of arabinose**

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14

15 **The L-arabinose-responsive AraC and its cognate P_{BAD} promoter underlie one of**
16 **the most often used chemically inducible prokaryotic gene expression systems in**
17 **microbiology and synthetic biology. Here we change the sensing capability of**
18 **AraC from L-arabinose to blue light, making its dimerization and the resulting**
19 **P_{BAD} activation light-inducible. We engineer an entire family of blue light-**
20 **inducible AraC dimers in Escherichia coli (BLADE) to control gene expression in**
21 **space and time. We show that BLADE can be used with pre-existing L-arabinose-**
22 **responsive plasmids and strains, enabling optogenetic experiments without the**
23 **need to clone. Furthermore, we apply BLADE to control with light the catabolism**
24 **of L-arabinose thus externally steering bacterial growth with a simple**
25 **transformation step. Our work establishes BLADE as a highly practical and**
26 **effective optogenetic tool with plug-and-play functionality—features that we hope**

27 **will accelerate the broader adoption of optogenetics and the realization of its vast**
28 **potential in microbiology, synthetic biology and biotechnology.**

29

30

31 Chemically inducible gene expression systems are invaluable tools to control biological
32 processes for basic science as well as biotechnological applications. While allowing for
33 tunability^{1, 2} and for some degree of spatial control³, these systems have some
34 limitations, namely they do not enable sophisticated spatio-temporal regulation and
35 often lack reversibility or require washing steps to achieve it. These limitations can be
36 overcome using light instead of small molecules as external trigger. For instance light
37 allows generating pulsatile inputs that alternate between dark (OFF) and maximum
38 intensity (fully ON), which have been shown to lead to effects that cannot be realized
39 with light of graded intensity, such as reduced cell-to-cell variability in gene
40 expression⁴. In fact, by adjusting the duty-cycle (defined as the fraction of the time that
41 the light is fully ON), the amount of cell-to-cell variability can be tuned, providing a
42 new control modality for studying stochasticity in gene expression. This type of
43 pulsatile input was also recently shown to enhance the biosynthesis of products in
44 engineered cells, enabling a new manner of bioreactor operation in which enzyme
45 expression is tuned to increase fermentation yield⁵.

46

47 Several light-inducible gene expression systems are available for use in bacteria⁶⁻¹²,
48 some of which featuring extremely high dark/light fold changes^{10, 11}. All of these tools
49 require the usage of a specific promoter. Here we aimed to harvest the well-known and
50 pervasive P_{BAD} promoter to allow optogenetic experiments to be performed with pre-
51 existing plasmids and strains *without the need for cloning*.

52 The P_{BAD} promoter regulates the *araBAD* operon encoding three enzymes that convert
53 the sugar L-arabinose to D-xylulose-5-phosphate^{13, 14}. In the absence of L-arabinose,
54 P_{BAD} is repressed by the transcriptional regulator AraC bound to the distal I_1 and O_2
55 half-sites, which causes the formation of a DNA loop that sterically blocks the access
56 of the RNA polymerase to the promoter (Fig. 1a). In the presence of L-arabinose,
57 transcription from the P_{BAD} promoter is activated by AraC, which additionally
58 negatively feeds back on its own promoter P_C ^{13, 14}. Activation results from AraC
59 binding to the adjacent I_1 and I_2 half-sites, which recruits the RNA polymerase (Fig.
60 1a). AraC is composed of an N-terminal dimerization domain (DD) and a C-terminal
61 DNA binding domain (DBD) connected via a linker (Fig. 1b). Interestingly, AraC is
62 always a homodimer, whether bound to arabinose or not¹⁴. Binding of arabinose
63 triggers a conformational change in AraC, which results in the two DBDs being
64 oriented in a way that favors their interaction with the I_1 and I_2 half-sites rather than the
65 I_1 and O_2 half-sites (Fig. 1a)^{13, 14}.

66 In this work, we engineer an entire family of Blue Light-inducible AraC Dimers in *E.*
67 *coli* (BLADE). After characterizing BLADE in terms of kinetics, reversibility, spatial
68 control, and light dependence, we demonstrate that BLADE can be used to regulate
69 with light previously constructed plasmids and strains obtaining reversibility that
70 cannot be achieved with L-arabinose. We show that BLADE functions by contacting
71 the I_2 half-site within the P_{BAD} promoter in the lit state, and that in the dark state
72 aggregates are formed, which might contribute to the tightness of the system. Finally,
73 we use BLADE to control the endogenous arabinose operon and direct *E. coli* growth
74 on L-arabinose with blue light.

75 We envision that BLADE will stimulate the incorporation of optogenetic experiments
76 in microbiology and will facilitate optogenetic endeavors in synthetic biology due to its

77 compatibility with previously constructed strains and plasmids, its added functionality
78 that cannot be easily achieved with chemical inducers, and its reliable performance.

79

80 **Results**

81 **Creation of chimeric VVD-AraC fusion constructs**

82 Inspired by a previous study in which chimeric AraC constructs have been cloned to
83 probe the role of the DD and DBD¹⁵, we reasoned that, by exchanging the dimerization
84 domain of AraC with a light-inducible dimerization domain (Fig. 1b), we would be able
85 to control with light the switching of this engineered AraC from monomer to dimer
86 (Fig. 1c). In its monomeric form, the engineered AraC would contact the high-affinity
87 I₁ half-site¹⁶, but not the low-affinity I₂ half-site, needed to recruit the RNA polymerase.
88 Its function as a light-inducible TF would depend on finding the appropriate linker
89 supporting the correct orientation of the two DBDs after dimer formation, permissive
90 of I₁-I₂ binding (Fig. 1c). As the light-triggered dimerization domain we selected at first
91 VVD, which has often been successfully employed to control with light the
92 dimerization of proteins of interest¹⁷⁻²⁰. VVD senses blue light *via* the flavin adenine
93 dinucleotide (FAD) chromophore²¹. Blue light triggers the formation of a cysteinyl-
94 flavin adduct, which generates a new hydrogen bond network that releases the N-
95 terminus (N-terminal cap) from the protein core and restructures it creating a new
96 dimerization interface^{22, 23}. We swapped the AraC dimerization domain with
97 VVD^{N56K/C71V}, a double mutant shown to stabilize the dimer¹⁸, and cloned seven
98 constructs having different linkers between AraC(DBD) and VVD as well as one
99 construct without linker (Fig. 1d). We removed the *araC* gene from pBAD33 and
100 introduced two constitutive promoters of different strength (J23101* and J23101**) to
101 drive the expression of the chimeric VVD-AraC(DBD) fusion constructs

102 (Supplementary Fig. 1). For a reporter gene, we cloned *mCherry* downstream of the
103 P_{BAD} promoter (Fig. 1e). As a positive control, we constructed the same plasmid
104 carrying full-length AraC in place of the VVD-AraC fusion (Supplementary Fig. 2a
105 and Supplementary Table 1), while the plasmid without any TF (pReporter_only) was
106 constructed to serve as negative control to monitor leaky expression from P_{BAD}
107 (Supplementary Fig. 2b and Supplementary Table 1). Flow cytometry analysis of *E.*
108 *coli* MG1655 cells transformed with the small library of VVD-AraC fusions, as well as
109 the negative and the positive controls, kept in the dark or illuminated with 460 nm light
110 (5 W/m²) for 4 hours showed that all 14 VVD-AraC constructs having a linker between
111 the two domains were light-inducible, despite being less optimal than full-length AraC
112 (Fig. 1f). The fusion without linker (FP8) did not activate gene expression regardless
113 of its expression levels (Fig. 1f). Different linkers corresponded to different amounts of
114 gene expression, with the longest linker being the least active. With the weaker
115 constitutive promoter driving expression of the VVD-AraC(DBD) fusion constructs
116 (J23101*), the levels of reporter expression in the dark approached those of the negative
117 control, to which the values were normalized (Fig. 1f). The stronger constitutive
118 promoter (J23101**) led to significantly higher expression of the reporter gene after
119 blue light illumination for all constructs, albeit at the cost of increased leakiness in the
120 dark (Fig. 1f). Nonetheless, for some of the fusions, the light/dark fold change was
121 higher with this promoter. We named a generic member of this family of Blue Light-
122 inducible AraC Dimers in *E. coli* BLADE and the pBAD33-derived corresponding
123 expression plasmid pBLADE (Fig. 1e). Reporter gene expression can be tuned not only
124 by selecting different promoters to drive the expression of BLADE (Fig. 1f), but also
125 by applying different light intensities (Fig. 1g). Importantly, even the highest intensity
126 used in our experiments (5 W/m²) is perfectly tolerated by the bacterial cells, thus it can

127 be safely used (Supplementary Fig. 3). To demonstrate that BLADE is useful to control
128 the expression of functional *E. coli* proteins, and not only fluorescent reporters, we
129 cloned several genes coding for proteins involved in cell division (MinD^{24, 25} and its
130 mutant MinD Δ 10²⁶) and cell shape (MreB²⁷ and RodZ²⁸) in place of mCherry into
131 pBLADE. We transformed each construct into MG1655 *E. coli* cells and either exposed
132 cells to 4 hours of blue light illumination or kept them in the dark. In all cases, cells
133 kept in the dark were indistinguishable from those transformed with pReporter_only,
134 which served as a negative control (Extended Data Fig. 1, Supplementary Fig. 4 and
135 Supplementary Video 1), demonstrating the tightness of BLADE. Light-induced
136 overexpression of the selected proteins caused the expected phenotypes, while light
137 itself had no effect (Extended Data Fig. 1).

138 While the conventional L-arabinose-responsive AraC is better than BLADE in terms of
139 fold change (Fig. 1f and Extended Data Fig. 2a,b), this latter benefits from the fact that
140 light is not catabolized (Extended Data Fig. 2b) and that its *utilization* is not dependent
141 on a series of cellular processes such as transport, thus leading to less heterogeneity
142 within the population especially at lower inductions (Extended Data Fig. 2c,d and
143 Supplementary Fig. 5). Moreover, light can be easily removed, while arabinose is not
144 easily washed out from the cells (Extended Data Fig. 2e,f and Supplementary Fig. 6).

145

146 **Spatial control of gene expression**

147 One of the benefits of optogenetic induction is the ability to spatially modulate gene
148 expression. To showcase how BLADE could be used to control the expression of a
149 target gene only in selected cells, we cloned sfGFP²⁹ into pBLADE. *E. coli* MG1655
150 cells transformed with pBLADE-sfGFP were then applied to an agar pad and subjected
151 to confocal microscopy to expose a limited area (6.4 μm^2) to blue light every 5 minutes.

152 After 3 hours, sfGFP was expressed up to 6.7-fold more in the illuminated cells
153 compared to the surrounding non-illuminated cells (Supplementary Fig. 7). Another
154 interesting application of light-inducible TFs that relies on the possibility to shine light
155 on a plate in desired patterns, is bacterial photography³⁰. To assess the effectiveness of
156 BLADE in this type of application, we covered one lawn of *E. coli* MG1655 cells
157 transformed with pBLADE-sfGFP with a photomask depicting the Blade Runner movie
158 poster (Fig. 2a) and another one with the photomask reproducing Michelangelo's
159 "Creation of Adam" fresco (Supplementary Fig. 8). We illuminated the plates with blue
160 light overnight and then took several microscopy pictures and stitched them together
161 (Fig. 2b,d). The sensitive light response of BLADE yielded a good contrast, resulting
162 in high quality bacteriographs that allowed for the faithful reproduction of the details
163 in the images, such as facial expressions (Fig. 2c).

164

165 **BLADE is compatible with pre-existing plasmids and strains**

166 The uniqueness of BLADE resides in its being based on the widely-used P_{BAD}
167 promoter. If BLADE outcompeted AraC for the activation of the pBAD promoter in
168 the absence of arabinose when AraC assumes the pBAD-inhibiting conformation (Fig.
169 1a), previously constructed plasmids and strains could be employed to perform
170 optogenetic experiments with a simple (co)transformation step to bring BLADE into
171 the cells (Fig. 3a). We therefore cloned the BLADE-encoding gene into the well-known
172 pTrc99a plasmid, into which we replaced the IPTG-inducible pTrc promoter with the
173 constitutive J23101** one (giving rise to pBLADE^{ONLY_A}; Supplementary Table 1).
174 We then co-transformed pBLADE^{ONLY_A} and a previously constructed pBAD33-
175 mCherry plasmid into MG1655 cells and compared the mCherry levels in this strain
176 with those in MG1655 cells co-transformed with pBLADE-mCherry and an empty

177 pTrc99a, allowing for the usage of the same two antibiotics. The mCherry reporter was
178 activated with blue light in both strains, albeit the fold change was slightly lower when
179 BLADE was used with a pre-existing pBAD33 due to the competition with apo-AraC
180 (Fig. 3b).

181 Next, we took a previously constructed strain (KC717), where the endogenous
182 promoter driving the expression of the *rodZ* gene has been exchanged with P_{BAD}³¹. In
183 the absence of arabinose, the endogenous chromosomal copy of AraC inhibits
184 transcription from P_{BAD}, thus RodZ is not expressed and cells are spherical^{28, 31-33}. In
185 the presence of arabinose, endogenous AraC initiates transcription from P_{BAD} and,
186 consequently, RodZ is expressed, leading to the reappearance of rod-shaped cells³¹. We
187 transformed KC717 cells either with a modified pBLADE from which the P_{BAD}
188 promoter and the mCherry gene were eliminated (pBLADE^{ONLY-C}; population A;
189 Supplementary Table 1) or with an empty pBAD33 deprived of *araC* and P_{BAD}, which
190 was used to allow growing both strains in the presence of the same antibiotic (pCAM;
191 population B; Supplementary Table 1) and kept both populations either uninduced (in
192 the dark for population A, and without arabinose for population B) or induced them for
193 4 hours (with blue light for population A and with arabinose for population B). At this
194 time point, population A recovered the rod shape to a greater extent than population B
195 (Fig. 3c). To showcase the power of optogenetics to quickly switch induction off, we
196 subjected the cells to a recovery phase, by putting them into the dark (population A)
197 and washing arabinose off (population B). While it was possible to obtain spherical
198 cells again after 2 hours of dark incubation, the cells that had been induced with
199 arabinose did not recover the initial phenotype and rather became even more rod-shaped
200 (Fig. 3c).

201

202 **BLADE aggregates in cells kept in the dark**

203 Wild-type AraC and BLADE are substantially different in their mode of action: AraC
204 is always a dimer that, in the absence of arabinose, binds the I₁ and O₂ half-sites and,
205 in the presence of the sugar, binds the I₁ and I₂ half-sites (Fig. 1a). In contrast, BLADE
206 is monomeric in the dark and dimeric under blue light illumination (Extended Data Fig.
207 3a,b). While the conformation assumed by dimeric BLADE cannot be easily predicted,
208 we know that it is able to contact the I₂ half-site, which is crucial for the recruitment of
209 the RNA polymerase. Indeed, mCherry was not expressed in an illuminated sample in
210 which the pBLADE plasmid was modified so that the I₂ half-site was in inverse
211 orientation while the -35 region of the P_{BAD} promoter was left unchanged (Extended
212 Data Fig. 3c,d).

213 To test if BLADE is degraded in the dark in *E. coli* as shown for VVD in *Neurospora*
214 *crassa*³⁴⁻³⁶, we constructed a C-terminal sfGFP-fusion to BLADE. We observed no
215 difference between the steady-state GFP levels under both conditions (Extended Data
216 Fig. 4a). Assuming an equivalent constitutive rate of GFP production for cells,
217 irrespective of the light input, these results suggest there is no differential degradation
218 in the two conditions. Fluorescence microscopy, however, revealed the formation of
219 aggregates in half of the cells kept in the dark (Fig. 4a, and Extended Data Fig. 4b,c).
220 The aggregates are due to the VVD moiety in BLADE, because a mutant BLADE with
221 alanine in place of the adduct-forming cysteine (VVD^{C108A}) showed aggregates also
222 under blue light illumination (Fig. 4b and Extended Data Fig. 4c), while wild-type
223 AraC-sfGFP was cytoplasmic (Fig. 4c). Time-lapse fluorescence microscopy indicated
224 that the aggregates do not disperse under blue light illumination, but are instead
225 asymmetrically segregated during cell division (Supplementary Video 2). Newborn

226 cells contain either no foci or foci much smaller than those found in cells kept in the
227 dark (Supplementary Video 2).

228

229 **Expanding the family of BLADE TFs**

230 In principle, BLADE could have been designed using other light-inducible dimerization
231 domains. Moreover, the position of this domain with respect to the DBD of AraC may
232 not need to reflect that found in the wild-type protein. To test if other functional
233 combinations with different characteristics could be identified, we generated a much
234 larger set of samples for characterization. As a light-inducible dimerization unit, we
235 included not only VVD, but also the Light Oxygen Voltage (LOV) domain of
236 *Vaucheria frigida* Aureochrome1 (VfAu1)^{37, 38}, which is naturally found C-terminally
237 to a bZip DBD³⁸, and which, like VVD, homodimerizes upon blue light stimulation³⁹,
238 ⁴⁰. To assess the functionality of the chimeric transcription factors (cTFs), we used only
239 the P_{BAD} promoter (I₁-I₂ half-sites) and removed the upstream regulatory elements (O₁
240 and O₂ half-sites¹⁰). We systematically explored how the expression levels of the cTF
241 affected mCherry levels in the dark and after blue light illumination. To this aim, we
242 first used an IPTG-inducible promoter⁴¹ to achieve various levels of expression of the
243 cTFs, with the goal of finding the most appropriate expression level, which can
244 subsequently be fixed using a constitutive promoter that matches the IPTG-induced
245 transcription (Extended Data Fig. 5 and Supplementary Fig. 9). In principle, the optimal
246 output might be achieved at intermediate cTF concentrations (Extended Data Fig. 5a).
247 Here we defined as output the light/dark fold change; however, depending on the
248 application, other properties such as high output expression or low dark state might be
249 more relevant. To meet our experimental needs, we developed a new device that is
250 compatible with standard 96-well microtiter plates and that allows for fast high-

251 throughput characterization (Extended Data Fig. 6a). In addition, we could test the N-
252 and C-terminal positioning of the light-inducible dimerization unit, as well as different
253 linkers connecting the two domains (Extended Data Fig. 6b). Using *E. coli* strain
254 MG1655 $\Delta araCBAD \Delta lacIZYA \Delta araE \Delta araFGH$, we tested the constructs in the library
255 with variable order between AraC(DBD) and the light-inducible dimerization unit and
256 explored a wide range of IPTG concentrations, from no induction to a concentration of
257 2 mM. For all constructs, the highest fold change was reached at intermediate mCherry
258 expression levels (Fig. 5a). Placing AraC(DBD) at the C-terminus led to higher fold
259 changes for VVD-based constructs, mainly due to lower mCherry expression in the
260 dark (Fig. 5b). For VfAu1, the opposite was true (Fig. 5c). Next, we investigated the
261 effect of linker length on the cTFs. Based on the results obtained with the first library,
262 we placed AraC(DBD) C-terminally for the VVD-based constructs, and N-terminally
263 for those based on VfAu1. We selected a set of linkers from a previous report⁴².
264 Additionally, we cloned variants without a synthetic linker for each of the dimerization
265 domains. We found that synthetic linker lengths up to 7 and 9 amino acids gave rise to
266 the highest fold change for VVD and VfAu1, respectively (Fig. 5d,e). All these
267 functional fusions expand the family of BLADE TFs. IPTG-dose response curves for
268 all samples are shown in Extended Data Fig. 7 and 8.

269 We performed the same systematic characterization of BLADE family members using
270 a synthetic P_{BAD} promoter, where the weak affinity I_2 half-site was exchanged with a
271 second copy of the high affinity I_1 half-site (Supplementary Fig. 10), which is
272 insensitive to arabinose, as it is constitutively active when used with wild-type AraC⁴³.
273 Results with this promoter again showed highest fold changes at no or low IPTG
274 induction (Fig. 5f) and were consistent with those obtained with the synthetic P_{BAD}
275 promoter consisting only of the I_1 and I_2 half-sites, in which C- and N-terminal

276 AraC(DBD) showed the highest fold change for fusions with VVD and VfAu1,
277 respectively (Fig. 5g,h), although with higher maximal dark/light fold changes for the
278 same cTFs compared to that obtained with the I₁-I₂ synthetic promoter. High IPTG
279 concentrations led to toxic amounts of mCherry expression and were, therefore,
280 indistinguishable for dark and light induction in most cases. IPTG-dose response curves
281 for all samples are shown in Supplementary Figs. 11 and 12.

282

283 **Controlling the L-arabinose metabolic pathway with light**

284 AraC naturally controls the expression of genes that code for various proteins, such as
285 transporters and other enzymes, necessary for the utilization of L-arabinose as carbon
286 source^{14, 44, 45} (Fig. 6a). Since these genes are under the P_{BAD} promoter, we reasoned
287 that it would be possible to control L-arabinose catabolism with light in a strain depleted
288 of AraC with a single transformation step to bring BLADE into the cells. We
289 transformed two different BLADE versions bearing AraC(DBD) either N- or C-
290 terminally (AraC(DBD)::G₄S::VVD and VVD::G₆S::AraC(DBD)) in *E. coli*
291 MG1655Δ*araC* (Fig. 6b, upper right). As a control, we used the *E. coli*
292 MG1655Δ*araCBAD* strain (Fig. 6b, upper left), which should not be able to catabolize
293 L-arabinose due to the lack of the AraA-B-D enzymes. We prepared cultures in M9
294 medium containing trace amounts of amino acids (0.0004% casamino acids) and either
295 no or 2% L-arabinose, and incubated them at 37°C either in the dark or under saturating
296 blue light. Growth was measured at 40-minute intervals using a spectrophotometer (Fig.
297 6c,d). Without the *araBAD* operon, no growth was detected in medium without
298 arabinose after 18h incubation in either the dark or blue light for strains containing
299 either BLADE construct (Fig. 6b, lower left). In the presence of 2% L-arabinose, we
300 observed a very small increase in absorbance for both dark and light-induced samples.

301 This might be due to low levels of promiscuity for L-arabinose utilization by other
302 pathways. For the strain carrying the endogenous *araBAD* operon, there was no growth
303 without L-arabinose regardless of the presence of light (Fig.6b, lower right). In contrast,
304 when 2% L-arabinose was present in the medium, growth was observed for both
305 BLADE constructs for the illuminated samples (Fig. 6b, lower right). For BLADE with
306 AraC(DBD) located at the N-terminus, we observed a higher dark state growth
307 compared to that with the construct with C-terminal AraC(DBD), which confirms our
308 previous characterization using the fluorescent protein. Comparison with *E. coli*
309 MG1655 Δ *araCBAD* shows minimally increased growth for the dark expression
310 (Supplementary Fig. 13), confirming tightness of BLADE. Growth curves of individual
311 samples are shown in Supplementary Figs. 14-17.

312

313 We further investigated how different expression levels of BLADE would impact
314 growth on L-arabinose. Because of the altered growth conditions compared to the
315 previous experiments, we again titrated the expression of BLADE with IPTG. As
316 shown previously, the IPTG induction can ultimately be mapped to constitutive
317 promoters for inducer-free light control (Extended Data Fig. 5). For both constructs, an
318 intermediate IPTG concentration corresponded to the highest cell density (Fig. 6e and
319 Extended Data Fig. 9a).

320

321 Finally, we grew *E. coli* MG1655 Δ *araC* cells transformed with the two BLADE
322 constructs in a medium containing 2% L-arabinose with four IPTG concentrations, and
323 either kept the cultures in the dark or exposed them to seven different light intensities
324 (Fig. 6f and Extended Data Fig. 9b). The data show that, when using BLADE to control
325 L-arabinose utilization, bacterial growth can be tuned by adjusting the light intensity

326 even if the amount of arabinose is fixed. Growth curves of individual samples are
327 shown in Supplementary Figs. 18 and 19.

328

329 **Discussion**

330 AraC is among the best studied bacterial transcriptional regulators, and its cognate
331 promoter P_{BAD} is one of the most widely used in microbiology, biotechnology and
332 synthetic biology. We have developed BLADE, a family of AraC-derived TFs that
333 activate transcription from the P_{BAD} promoter in response to blue light instead of
334 arabinose. The uniqueness of BLADE as a light-inducible system lies in its
335 compatibility with previously constructed L-arabinose-inducible plasmids and strains
336 carrying the P_{BAD} promoter at an endogenous locus to drive the expression of a gene of
337 interest. This makes it possible to readily perform optogenetic experiments without the
338 need to construct anything new—a single transformation step is the only requirement
339 (Fig. 3). We also demonstrated this by using BLADE to precisely activate the
340 endogenous pathway for L-arabinose catabolism only with light (Fig. 6).

341

342 When the use of existing L-arabinose-inducible plasmids or strains is not paramount,
343 light induction can be achieved by employing pBLADE, a single plasmid bearing both
344 BLADE and the P_{BAD} promoter followed by a multiple cloning site into which a gene
345 of interest is cloned (Fig. 1e). The advantage of pBLADE compared to other previously
346 constructed plasmids is that we created it using pBAD33 as a template (Supplementary
347 Fig. 2a), thus the resistance cassette and origin of replication of pBLADE are identical
348 to those of pBAD33, which ensures compatibility with other previously constructed
349 plasmids that should be co-transformed with pBLADE.

350

351 Recently, another pervasive chemically inducible gene expression system, namely the
352 one based on the *lac* operon, has been turned into a light-inducible one⁴⁶. In this case,
353 however, the *lacI* repressor itself was not engineered to sense light; rather, its
354 expression was put under light control using the established pDawn optogenetic
355 transcription system⁶. Beyond the relatively large number of proteins required (four),
356 OptoLAC has inherent memory for a light input, which prohibits fast inactivation upon
357 light removal⁴⁶. While being an advantage under certain circumstances, this feature is
358 limiting when fast dynamics are needed. Unlike OptoLAC, BLADE does not require
359 additional circuitry for light inducibility, which is achieved in a direct and less complex
360 manner. In addition, BLADE offers a far more dynamic control over gene expression
361 due to its immediate response to a light input through dimerization.

362

363 An important feature of BLADE is its minimal leakiness. Often, leakiness has been
364 assessed by simply looking at the levels of reporter expression in the absence of the
365 stimulus. However, this does not take into account whether the expression in the
366 uninduced state is already too high compared to the expression in the absence of the
367 regulator. In the case of BLADE, minimal leakiness was demonstrated by comparing
368 its activity in the dark with expression obtained with the same plasmid deprived of the
369 TF (pReporter_only; Fig. 1f). We put BLADE to the test by expressing several
370 functional *E. coli* proteins whose overexpression causes morphological changes to the
371 cells and showed that, in the dark, cells are indistinguishable from the control (Extended
372 Data Fig. 1 and Supplementary Fig. 4). Minimal leakiness was also observed when
373 controlling growth on L-arabinose with BLADE, as samples kept in the dark grew only
374 slightly compared to the control cells lacking the pathway (Fig. 6b,e, and
375 Supplementary Fig. 13)

376 We also investigated the mechanism of BLADE-mediated gene expression and found
377 that it involves dimer formation under blue light and subsequent contacting of the I2
378 DNA half-site. Interestingly, we discovered that BLADE localizes to aggregates in the
379 dark in half of the cell population (Fig. 4a and Extended Data Fig. 4c), which we
380 speculate might contribute to its tightness.

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382 When engineering new TFs, we found that optimal functionality not only requires the
383 careful engineering of the light-sensitive TFs themselves, but also the proper calibration
384 of their concentration. In particular, we found that, if concentrations exceed certain
385 levels, the functionality of the TF may in fact deteriorate (Fig. 5a) or disappear
386 altogether (Fig. 5f). For the metric of light/dark fold change, intermediate TF
387 expression levels always led to the highest values. While we used IPTG to express a
388 wide range of BLADE protein concentrations for this characterization, it is possible to
389 have BLADE expressed constitutively at any of these concentrations for specific
390 applications (Fig. 1f and Extended Data Fig. 5).

391

392 Beyond its favorable features, BLADE has some limitations, too. The maximum
393 reporter gene expression level obtained with BLADE, while adequate for most
394 applications, is not nearly as high as that reached with endogenous AraC or some other
395 light-inducible systems^{6, 8, 10, 11}. Moreover, the off-rate of any LOV domain-based tool
396 is dictated by the time it takes for it to go back to the dark state and cannot be modulated
397 with a second wavelength, as for phytochromes and phytochrome-related photosensors.
398 Nevertheless, should faster off-dynamics be needed for specific applications, mutations
399 can be introduced to speed-up the dark state reversion of VVD⁴⁷.

400

401 Taken together, BLADE adds a valuable new tool with distinctive features to the
402 growing arsenal of optogenetic gene expression systems. We envisage that BLADE's
403 effectiveness as a transcriptional activator combined with its plug-and-play
404 functionality, ease of use, and low cost make it a compelling system that will contribute
405 to the widespread adoption of optogenetic tools in the fields of microbiology,
406 biotechnology and synthetic biology.

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533 **Acknowledgements**

534 We thank Maximilian Hörner for his help with the determination of the absorption
535 spectrum of BLADE, João Nuno de Sousa Machado for his help with size-exclusion
536 chromatography, Yanik Weber for help with characterizing the 96-well light induction
537 plate, KC Huang for sharing with us the KC717 strain, and Stephanie Aoki for helpful
538 discussions. This study was funded by the DFG (grant no. VE776/2-1 to B.D.V.), by
539 the BMBF (grant no. 031L0079 to B.D.V.), by the Excellence Initiative of the German
540 Federal and State Governments BIOSS (Centre for Biological Signalling Studies; EXC-
541 294), by the European Research Council (ERC-Advanced) under the European Union's
542 Horizon 2020 research and innovation programme (grant agreement number 743269),
543 and FET-Open research and innovation actions grant under the European Union's

544 Horizon 2020 research and innovation programme (CyGenTiG; grant agreement
545 801041).

546

547 **Author contributions**

548 B.D.V. and A.B. conceived the study. B.D.V and M.K. supervised the study, and
549 secured funding. E.R., A.B., E.A., N.P., M.K. and B.D.V. designed experiments and
550 interpreted the data. E.R., A.B., M.H. and E.A. performed *in vivo* experiments. N.P.
551 purified BLADE, and performed size-exclusion chromatography. L.E. performed
552 initial experiments, which validated the idea. G.S. developed the 96-well light setup in
553 collaboration with A.B. M.A.Ö. performed bioinformatics and computational structural
554 biology analyses of the BLADE constructs. E.R., A.B., M.K. and B.D.V. wrote the
555 manuscript.

556

557 **Competing interests**

558 The authors declare no competing interests.

559

560 **Figure legends**

561

562 **Fig. 1| Engineering and characterization of a novel light-inducible AraC a,**
563 Mechanism of arabinose-induced P_{BAD} induction by AraC. The thickness of the I₁ and
564 I₂ half-sites symbolizes the affinity with which AraC binds to them. P_C, promoter
565 driving the expression of *araC*. **b,** Domain composition of wild-type (left) and light-
566 inducible (right) AraC. **c,** Expected mechanism of P_{BAD} activation by light-inducible
567 AraC. **d,** Domain composition of the chimeric VVD-AraC DNA binding domain
568 (DBD) fusion constructs. In FP3, amino acids 171-178 of the natural linker are present
569 twice. **e,** Plasmid for expression of a gene of interest (here mCherry) under control of
570 BLADE. **f,** mCherry fluorescence intensity in *E. coli* MG1655 cells transformed with
571 the library shown in **(d)** grown for 4h either in the dark or under 460 nm light (5 W/m²)
572 illumination. Native AraC cloned under the same constitutive promoters was used as a
573 positive control (AraC_{WT}). The bars for AraC represent the values obtained without
574 (grey) and with 0.1% (orange) arabinose for 4h. **g,** mCherry fluorescence intensity
575 measured in *E. coli* MG1655 cells transformed with the FP6 fusion driven by the
576 J23101** promoter grown for 4h under 460 nm light of the indicated light intensity

577 (cyan) or kept in the dark for 4h (black). **f,g**, Values were normalized to the mCherry
578 fluorescence intensity measured in *E. coli* MG1655 cells transformed with
579 pReporter_only (see Supplementary Table 1; dashed line). The individual data points
580 are the mean values of 10,000 single-cell flow cytometry events. **f**, Values represent
581 mean \pm SD of $n=3$ (FP2*, FP3* FP5*, FP1**, FP7**, PC**), $n=4$ (FP1*, FP4*, FP7*,
582 FP8*, FP2**, FP3**, FP5**), $n=5$ (FP4**, FP8**, PC**), $n=6$ (FP6**), 7 (PC*), and
583 $n=9$ (FP6*) biological replicates acquired on more than three different days. **g**, Values
584 represent mean \pm SD of $n=3$ (dark), and $n=4$ (light) biological replicates acquired on
585 three different days.

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589 **Fig. 2| BLADE allows for the production of high contrast bacteriographs. a**,
590 Photomask used to produce the bacteriograph in **(b)** (printed with permission from
591 Warner Bros. Entertainment, Inc.). **b,d**, Bacteriographs. Two lawns of *E. coli* MG1655
592 cells transformed with pBLADE(FP6*)-sfGFP were grown overnight at 37 °C while
593 being exposed to blue light through the photosmask in **(a)** or Supplementary Fig. 8 (**b**
594 and **d**, respectively). Bacteriographs were performed multiple times with similar
595 results. 110 **(b)** and 160 **(d)** individual images were taken with a fluorescent microscope
596 and stitched together via the Zen Blue 2.3 software. Scale bar, 1 cm. **c**, Zoom in on two
597 parts of the bacteriograph shown in **(b)**. Scale bar, 300 μ m.

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601 **Fig. 3| BLADE is compatible with pre-existing L-arabinose-responsive plasmids**
602 **and strains. a**, Schematic representation of the way in which BLADE can be used to
603 control with light the expression of a gene of interest previously put under L-arabinose
604 control, either genomically or on a plasmid. **b**, Upper panel, representative DIC images
605 of *E. coli* KC717 cells transformed with the indicated constructs at the indicated time
606 points. Images were acquired on three independent days with similar results.
607 pBLADE^{ONLY_C}, pBAD33 deprived of the P_{BAD} promoter expressing only BLADE.
608 pCAM, empty pBAD33 deprived of the P_{BAD} promoter. Induction indicates 460 nm
609 light (5 W/m²) for the cells transformed with pBLADE^{ONLY_C} and 0.2% arabinose for
610 the cells transformed with pCAM. Recovery indicates darkness for the cells
611 transformed with pBLADE^{ONLY_C} and growth in a medium without arabinose for the
612 cells transformed with pCAM. Scale bar, 5 μ m. Lower panel, quantification of the cell
613 roundness for the samples and conditions in the upper panel. Roundness is defined as
614 $4 \times [\text{Area}] / (\pi \times [\text{Major axis}]^2)$. Values represent mean \pm SD of $n=3$ independent
615 experiments. From left to right: $P=0.05520$, $P=0.00001$, $P=0.00012$, $P=0.18321$,
616 $P=0.19252$, $P=0.00006$, and $P=0.00004$. Not significant (ns), $P>0.05$; triple asterisk
617 (***), $P<0.001$; quadruple asterisk (****), $P<0.0001$. P-values P were calculated by
618 the two-tailed, homoscedastic Student's t-test. **c**, mCherry fluorescence intensity in *E.*
619 *coli* MG1655 cells co-transformed with the indicated plasmids grown for 4h either in
620 the dark or under 460 nm light (5 W/m²) illumination. pBLADE^{ONLY_A}, pTrc99a
621 deprived of the pTrc promoter expressing only BLADE. All values were normalized to
622 the mCherry fluorescence intensity measured in *E. coli* MG1655 cells transformed with
623 pReporter_only (see Supplementary Table 1; dashed line). The individual data points
624 are the mean values of 10,000 single-cell flow cytometry events. Values represent mean
625 \pm SD of $n=8$ (pBLADE^{ONLY_A}+pBAD33-mCherry), and $n=6$ (pBLADE-

626 mCherry+pTRC99a) biological replicates acquired on three different days. **b,c**,
627 BLADE variant: FP6 driven by the J23101** promoter.

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630 **Fig. 4| BLADE-sfGFP forms aggregates in the dark.** Representative microscopy
631 images of *E. coli* MG1655 cells expressing the indicated construct grown for 4h in the
632 dark (**a,b**) or in a medium without arabinose (**c**), or under 460 nm light (5 W/m²) light
633 (**c,d**) or in a medium with 0.1% arabinose (**e**). Scale bar, 5 μm. Images were acquired
634 on three days with similar results.

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637 **Fig. 5| Engineering an optimized and expanded family of BLADE TFs. a**, Examples
638 of IPTG dose-response curves obtained with VVD::G4S::AraC(DBD) and
639 AraC(DBD)::G20S5::VfAu1. The highest light/dark fold change is indicated with a red
640 line. **b,c**, Reporter gene fluorescence obtained with VVD::G4S::AraC(DBD) (C-
641 terminal) and AraC(DBD)::G4S::VVD (N-terminal) (**b**) or
642 VfAu1::(G4S)₅::AraC(DBD) (N-terminal) and AraC(DBD)::(G4S)₅::VfAu1 (C-
643 terminal) (**c**) in the presence of 125 μM and 62.5 μM IPTG (**b**) or 125 and 31.25 μM
644 IPTG (**c**), respectively. The red box indicates the samples for which the dose response
645 curve is shown in (**a**). In Extended Data Figs. 7 and 8 we show the data obtained with
646 AraC(DBD)::G4S::VVD and VfAu1::(G4S)₅::AraC(DBD), respectively. **d,e**, Reporter
647 gene fluorescence obtained with a library of constructs with different linkers between
648 C-terminal AraC(DBD) and VVD (**d**) or N-terminal AraC(DBD) and VfAu1 (**e**). The
649 same IPTG concentration was used for all constructs (125 μM in (**d**) and 31.25 μM in
650 (**e**)). For IPTG dose-response curves see Extended Data Figs. 7 and 8. **f**, Same as in (**a**)
651 but with a synthetic P_{BAD} promoter containing two copies of the I₁ half-site. The highest
652 light/dark fold change is indicated with a red line. **g**, Same as in (**b**) but with a synthetic
653 P_{BAD} promoter containing two copies of the I₁ half-site. 7.8125 and 3.906 μM IPTG
654 were used to induce the C- and N-terminal fusion constructs, respectively. See
655 Supplementary Fig. 11 for the data obtained with AraC(DBD)::G4S::VVD. **h**, Same as
656 in (**c**) but with a synthetic P_{BAD} promoter containing two I₁ half-sites. 15.625 μM and
657 no IPTG were used to induce the expression of the C- and N-terminal fusion constructs,
658 respectively. For the IPTG dose-response curve of VfAu1::G20S5::AraC(DBD) see
659 Supplementary Fig. 12. **g,h**, The samples in the red box are taken from the dose-
660 response curve shown in (**f**). All panels show mCherry fluorescence intensity of
661 MG1655 ΔaraCBAD ΔlacIZYA ΔaraE ΔaraFGH cells grown for 5h either in the dark
662 or under 465 nm light illumination (3.85 W/m²). Values represent mean ± SD of
663 n=3,4,5 biological replicates acquired separately in time.

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667 **Fig. 6| Growth on L-arabinose can be controlled with light using BLADE TFs. a**,
668 Schematic representation of the operon involved in L-arabinose catabolism in *E. coli*
669 and the principle behind BLADE-mediated control of the pathway. **b**, Upper panel,
670 schematic representation of the strains and plasmid used. Lower panel, optical density
671 of *E. coli* MG1655ΔaraCBAD cells transformed with AraC(DBD)::G4S::VVD (N-
672 terminal AraC(DBD)) and VVD::G6S::AraC(DBD) (C-terminal AraC(DBD)) (left) and
673 *E. coli* MG1655ΔaraC transformed with the same constructs (right) after incubation
674 for 18h at 37°C either in the dark or under 465 nm light illumination (3.85 W/m²). Cells
675 were grown in M9 medium spiked with 0.0004% CAS amino acids without (-) or with

676 2% L-arabinose (+). All samples expressing the N-terminal fusion were induced with
677 62.5 μM IPTG, while those expressing the C-terminal fusion were induced with 125
678 μM IPTG. **c,d**, Growth curves of *E. coli* MG1655 ΔaraC cells expressing
679 AraC(DBD)::G₄S::VVD (N-terminal AraC(DBD)) (**c**) or VVD::G₆S::AraC(DBD) (C-
680 terminal AraC(DBD)) (**d**) in a medium containing 2% arabinose and 62.5 (**c**) or 125
681 (**d**) μM IPTG at 37°C either in the dark or under 465 nm light (3.85 W/m²). Red dashed
682 line, time point at which growth values are shown in (**b**). **e**, Optical density of *E. coli*
683 MG1655 ΔaraC cells expressing VVD::G₆S::AraC(DBD) grown in a medium
684 containing 2% arabinose and the indicated IPTG concentrations grown for 18h at 37°C
685 either in the dark or under 465 nm light (3.85 W/m²). **f**, Optical density of *E. coli*
686 MG1655 ΔaraC cells expressing VVD::G₆S::AraC(DBD) grown in a medium
687 containing 2% arabinose and the indicated IPTG concentrations grown for 17h20' at
688 37°C either in the dark or under 465 nm light of the indicated intensity. Values represent
689 mean \pm SD of $n=3$ biological replicates acquired separately in time. Each data point
690 shown in the bar plots represents a data point in a time course with measurements every
691 40 min, of which the average of the first three measurements was subtracted from all
692 following time points to adjust for small differences in sample volume and inoculum.
693 All time courses are shown in Supplementary Figs. 14-17, and 19.
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695

696 **Methods**

697 **Strains, media and reagents.** The strains used in this study are listed in Supplementary
698 Table 2. For experiments shown in Figs. 1, 3b, 4, Extended Data Figs. 1d,e,f, 2 and 4,
699 and Supplementary Figs. 3, 5, 6, the cultures were grown in autoclaved Tryptone Broth
700 (TB; 10 g l⁻¹ Tryptone, 5 g l⁻¹ NaCl, 1 mM NaOH). For the experiments shown in Figs.
701 2, 3c and Extended Data Fig. 1b,c the cultures were grown in autoclaved LB-Miller
702 medium. For experiments shown in Figs. 5 and 6, Extended Data Figs. 5, 7 and 8, and
703 Supplementary Figs. 11 and 12, the cultures were grown in autoclaved LB-Miller
704 medium for strain propagation and in sterile-filtered M9 medium supplemented with
705 0.2% casamino acids, 0.4% glucose, 0.001% thiamine, 0.00006% ferric citrate, 0.1 mM
706 calcium chloride, 1 mM magnesium sulfate for all gene expression experiments. For
707 experiments shown in Supplementary Fig. 7 and Supplementary Video 2 the cultures
708 were grown in tethering buffer (10 mM potassium phosphate, 0.1 mM EDTA, 1 mM
709 L-methionine and 10 mM sodium lactate; pH 7.0). In experiments in which plasmids

710 had to be maintained, the medium was supplemented with either 34 $\mu\text{g ml}^{-1}$
711 chloramphenicol or 100 $\mu\text{g ml}^{-1}$ ampicillin and 34 $\mu\text{g ml}^{-1}$ chloramphenicol (Sigma-
712 Aldrich Chemie GmbH). IPTG, rifampicin and doxycycline were purchased from
713 Sigma-Aldrich Chemie GmbH.

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715 **Constructions of strains and plasmids.** To integrate *lacY* expressed from the *rrnB P1*
716 promoter into the *attB* site of BW25113^{48,49} and to delete *araC* and *araBAD* we used λ
717 integrase expressed from pJW27⁵⁰. The corresponding DNA containing a FRT-flanked
718 *kanR* from pKD13 for selection was transformed as linear DNA into *E. coli* BW25113
719 or MG1655 K-12 (ATCC 47076)⁵¹ and selected at 30°C on LB-Agar plates containing
720 chloramphenicol for expression of λ integrase. A single colony was used to inoculate 5
721 ml of LB broth containing chloramphenicol, and the culture was grown at 30°C in a
722 water bath with shaking. The cells were then moved to 42°C for 15 min, before
723 incubation on ice for 15 min.

724 Cloning was performed via NEBuilder® HiFi DNA Assembly (New England Biolabs)
725 or restriction enzymes. Detailed information of all the primers, backbones and inserts
726 used in the cloning can be found in the Supplementary Dataset 1. All PCRs were
727 performed using the Phusion Flash High Fidelity PCR Master Mix (Thermo Scientific).
728 Oligonucleotides were ordered at Sigma Aldrich. Ligation reactions were transformed
729 into chemically competent *E. coli* TOP10 cells.

730 To clone the extended library of BLADE TFs, we used a modular Golden Gate cloning
731 strategy using an optimized junction set for part assembly taken from⁵². The overhangs
732 as well as the individual parts and the final plasmid sequences are shown in
733 Supplementary Dataset 2 and 3. To invert the transcriptional unit containing the
734 *mcherry* gene under AraC-controlled promoters, we first assembled the transcriptional

735 unit separately, and then PCR-amplified the resulting fragment to create an A junction
736 inverted at the end, and an F junction inverted at the beginning of the transcriptional
737 unit and further treated the resulting construct as a part. Individual parts were first
738 cloned into a part vector using BbsI-HF. The final plasmids were assembled from
739 individual parts with BsaI-HF for digestion of the parts, and BbsI-HF for digestion of
740 the plasmid backbone, which contains a p15a and a chloramphenicol acetyl transferase.
741 Plasmids were transformed using a one-step preparation protocol of competent *E. coli*
742 cells for transformation of plasmids in testing strains⁵³. The sequences of all cloned
743 plasmids were confirmed by Sanger sequencing (Eurofins Genomics Europe
744 Sequencing GmbH, Köln, Germany, and Microsynth AG, Balgach, Switzerland). A list
745 of all vectors used and constructed in this study is shown in Supplementary Table 1 and
746 Supplementary Dataset 3. Oligonucleotide sequences used for PCR amplification and
747 Golden Gate part sequences are shown in Supplementary Dataset 2 and 4. The cloning
748 was performed using chemically competent *E. coli* TOP10 cells (Thermo Scientific).

749

750 **Bacterial growth.** For experiments shown in Figs. 1-4, Extended Data Fig. 1-4 and
751 Supplementary Figs. 3-7, cultures were handled under safe red light whenever
752 containing light-sensitive constructs. The cultures were incubated overnight in TB or
753 LB medium and grown at 37°C in an incubator shaking at 250 rpm, in black plastic
754 tube (Argos Technologies LiteSafe® 15 ml) if containing light-sensitive samples, in
755 transparent glass tubes otherwise. The following morning, the cultures were diluted to
756 $OD_{600} = 0.1$ and let grow until $OD_{600} = 0.4$. Half of the culture was then transferred in
757 transparent glass tubes and induced either with blue light or with arabinose for 4 hours.
758 For experiments shown in Figs. 5 and 6, Extended Data Figs. 5, 7 and 8, and
759 Supplementary Figs. 11-19, cultures were grown in an environmental shaker. The

760 shaking incubator consisted of a Kuhner ES-X shaking module (Adolf Kühner AG,
761 Basel, Switzerland) mounted inside an aluminum housing (Tecan, Maennedorf,
762 Switzerland) and temperature-controlled using an “Icecube” (Life imaging services,
763 Basel, Switzerland). Cultures were grown at 37°C with shaking at 300 rpm in black,
764 clear-bottom 96-well plates (Cell Culture Microplates 96 Well μ Clear® CELLSTAR®,
765 Greiner Bio-One GmbH, Product #: 655090), which were sealed with pierceable foil
766 (Sealing foil, clear pierceable thin seal for PlateLoc, No. 17318-001, Agilent) for
767 fluorescent reporter expression and peelable foil (Sealing foil, clear peelable for
768 PlateLoc, No. 16985-001, Agilent) for growth experiments to prevent liquid
769 evaporation and guarantee sterility, as well as a plastic lid (Greiner Bio-One GmbH,
770 Product #: 656171). Overnight cultures were inoculated in M9 medium and grown over
771 night to an OD₆₀₀ of about 4. These cultures were diluted 1:20,000 into fresh M9
772 medium containing the respective inducer concentrations, right before the start of the
773 experiment. This high dilution ensures that the cells are still in logarithmic growth
774 phase after 5h, at the end of the experiment⁵⁴. 200 μ l of inoculated culture were
775 incubated per well in the 96-well plates. Cells were grown for 5h before transcription
776 and translation was stopped with rifampicin and tetracycline¹⁰. The inhibition solution
777 contained 500 μ g ml⁻¹ rifampicin and 50 μ g ml⁻¹ tetracycline in phosphate buffered
778 saline (Sigma-Aldrich Chemie GmbH, Dulbecco’s phosphate buffered saline) and was
779 filtered using a 0.2 μ m syringe filter (Sartorius). 100 μ l inhibition solution were
780 aliquoted in 96-well U-bottom plates (Thermo Scientific Nunc), precooled on ice and
781 samples were added in equal volumes (100 μ l), resulting in a final inhibitor
782 concentration of 250 μ g ml⁻¹ rifampicin (Sigma-Aldrich Chemie GmbH) and 25 μ g ml⁻¹
783 tetracycline (Sigma-Aldrich Chemie GmbH). After sample was added, the solution
784 was incubated on ice for at least 30 min. Then mCherry maturation was carried out at

785 37 °C for 90 min. The samples were kept at 4°C until measurement through flow
786 cytometry.

787

788 **Light illumination systems.** To illuminate the glass tubes in the shaker, six high-power
789 460 nm LEDs type CREE XP-E D5-15 (LED-TECH.DE) were used (Supplementary
790 Fig. 20). The LEDs were connected to a power supply (Manson HCS-3102) that
791 allowed to tune the voltage, hence the light intensity. Unless specified, the light
792 intensity reaching the cultures was 5 W/m² as measured with a LI-COR LI-250A Light
793 Meter. For the bacterial photography, we used a custom-made light box with, among
794 others, 6 blue (455 nm) LEDs (Supplementary Fig. 21). To avoid generation of a
795 blurred image in the bacteriograph, all the LEDs except for the one in the center were
796 obscured with colored tape. The average light intensity reaching the plate was 1.3
797 W/m².

798 Although another light plate was available⁵⁴, we designed a new 96-well plate to meet
799 our experimental requirements. The 96-LED array was designed using CircuitMaker
800 1.3.0 (www.circuitmaker.com). The LEDs (SK6812, Dongguan Opsco
801 Optoelectronics Co., Dongguan City, China) were arranged on the PCB at a pitch of 9
802 mm in an 8 x 12 grid to be compatible with standard 96-well plates. All LEDs were
803 daisy-chained using their DIN and DOUT ports. A 0.1 nF capacitor was placed in
804 parallel to the VDD port of each LED as proposed by the manufacturer. The 2-layer
805 circuit was manufactured on a 1.6 mm thick FR-4 substrate, and the surface of the PCBs
806 was coated with black solder mask to reduce reflection. The PCBs were ordered
807 preassembled with the LEDs and 0.1 nF capacitors (www.pcbway.com, Shenzhen,
808 China). Every 96-LED PCB had one signal-in and one signal-out SMA connector such
809 that several 96-LED PCBs could be daisy-chained using SMA cables and controlled by

810 a single microcontroller. Up to 4x 96-LED PCBs could be powered using a single
811 Adafruit #658 5 V 10 A switching power supply (digkey.ch, Munich, Germany) using
812 a custom-made PCB to distribute the power to several LED arrays. The LEDs were
813 controlled through an Arduino Uno microcontroller (Arduino, Somerville, MA, USA)
814 using the fastLED library (<http://fastled.io/>).

815 The 96-LED array was mounted inside the shaking incubator using custom 3D-printed
816 holders. The holders were printed with an Ultimaker S5 using black Ultimaker CPE
817 (Ultimaker, Utrecht, Netherlands) to reduce reflections. For better dissipation and
818 distribution of the heat generated by the LEDs, a custom-made anodized aluminum
819 plate (10 mm thick, with 96 holes of 4 mm diameter) was mounted on top of the 96-
820 LED array. Another 3D-printed adapter was placed between the aluminum plate and
821 the microtiter plate to ensure optical insulation of the wells. The 3D-printed parts and
822 the metal plate were aligned and held in place by metal rods (4 mm diameter, 20 mm
823 length).

824

825 **Flow Cytometry.** For experiments shown in Figs. 1, 3, Extended Data Figures 1-4 and
826 Supplementary Figs. 3, 5, 6, fluorescence was measured using the LSR Fortessa flow
827 cytometer (BD Biosciences). Samples were centrifuged at 4000 *g* for 4 min to remove
828 the glycerol-containing solution, then the pellets were resuspended in PBS. Data
829 analysis was performed using the open source FCSalyzer software (V 0.9.1.5 α). This
830 software was also used to calculate the mean fluorescence and the coefficient of
831 variation (CV) for each sample. The mCherry fluorescence was excited with a 561 nm
832 laser (50 mW), and emission was detected using a 610/20-nm filter pass (PMT voltage
833 set to 750 V). The GFP fluorescence was excited with 488 nm laser (100 mW), and
834 emission was detected using a 530/30-nm filter pass (PMT voltage set to 405 V). A

835 forward scatter height (FSC-H) threshold of 1,400 was used to gate for living cells and
836 eliminate debris. 10^5 events per sample were recorded for each experiment. The cell
837 density of the samples was manually regulated by addition of PBS in order to have less
838 than 2×10^4 events/s recorded by the machine. To compensate any variable that can alter
839 the measurement of the fluorescence by the flow cytometer, each experiment was
840 normalized with the fluorescence value of the negative control grown the same day of
841 the experiment. For experiments shown in Fig. 5, Extended Data Figs. 5, 7 and 8 and
842 Supplementary Figs. 11 and 12, fluorescence was measured on a Cytoflex S flow
843 cytometer (Beckman Coulter) equipped with CytExpert 2.1.092 software. The mCherry
844 fluorescence was excited with a 561 nm laser and emission was detected using a 610/20
845 nm band pass filter and following gain settings: forward scatter 100, side scatter 100,
846 mCherry gain 700 when mCherry was expressed from the I₁-I₂ promoter, and 100 gain
847 when mCherry was expressed from the I₁-I₁ promoter due to the difference in
848 expression levels. Thresholds of 2,500 FSC-H and 1,000 SSC-H were used for all
849 samples. The flow cytometer was calibrated before each experiment with QC beads
850 (CytoFLEX Daily QC Fluorospheres, Beckman Coulter) to ensure comparable
851 fluorescence values across experiments from different days. All flow cytometry data
852 containing the weaker expressing I₁-I₂ half sites contains more than 15000 events in
853 the used gate per individual sample. All flow cytometry data containing the stronger
854 expressing I₁-I₁ half sites had more than 15000 events in the used gate per individual
855 sample until IPTG concentration 15.625 μ M, which covers all data that we drew
856 conclusions from. The count number for samples with IPTG concentrations higher than
857 15.625 μ M using the I₁-I₁ half sites decreased due to slower growth caused by toxicity
858 of high gene expression, but in all cases more than 500 counts in the used gate per
859 individual sample were collected. We used a two-dimensional forward and side scatter

860 gate, which was drawn by eye and corresponded to the experimentally determined size
861 of the testing strain at logarithmic growth and was kept constant for analysis of all
862 experiments and used for calculations of the median and CV using the CytExpert
863 software. The same gating strategy was previously used and is depicted in Extended
864 Data Fig. 10.

865

866 **Characterization of the FP1-FP8 VVD-AraC(DBD) fusion constructs.** Overnight
867 cultures of cells transformed with the FP1-FP8 fusions as well as the controls were
868 diluted to $OD_{600} = 0.1$, let grow in the dark to $OD_{600} = 0.4$ and split into two cultures,
869 one of which was kept in the dark and one of which was illuminated for 4 h. The
870 overnight culture of the negative control was diluted to $OD_{600} = 0.1$, and let grow for
871 the same amount of time as all other cultures (circa 5 h 30 min). The overnight cultures
872 of the positive controls were diluted to $OD_{600} = 0.1$, let grow to $OD_{600} = 0.4$ and split
873 into two cultures, one of which was left without arabinose and one of which was
874 induced with 0.1% arabinose for 4 h. After the induction time, 200 μ l of each sample
875 were collected, mixed with 200 μ l of a transcription and translation inhibition solution
876 ($500 \mu\text{g ml}^{-1}$ rifampicin and $50 \mu\text{g ml}^{-1}$ doxycycline in phosphate buffered saline) and
877 incubated in the dark 90 min at 37°C with 110 rpm shaking. This protocol allows
878 obtaining a full maturation of almost all the mCherry proteins translated at the end of
879 the induction time¹. After the incubation with the inhibitor, samples were diluted 1:1
880 with 60% glycerol and frozen at -80°C .

881

882 **Dynamic control of gene expression.** The overnight cultures transformed with
883 pBLADE(FP6**)-mCherry, pReporter_only (negative control) and pBAD33-mCherry
884 were diluted in TB to $OD_{600} = 0.05$ in dark tubes and glass transparent tubes,

885 respectively, and let grow until $OD_{600} = 0.15$. 200 μl of each sample were collected,
886 mixed with 200 μl of a transcription and translation inhibition solution (500 $\mu\text{g ml}^{-1}$
887 rifampicin and 50 $\mu\text{g ml}^{-1}$ doxycycline in phosphate buffered saline), incubated in the
888 dark 90 min at 37°C with 110 rpm shaking, diluted 1:1 with 60% glycerol, and frozen
889 at -80°C. The rest of the culture was transferred in transparent glass tubes: the culture
890 with BLADE was illuminated with blue light as described (see “Light illumination
891 systems”) for 2 h, while the culture with pBAD33 was split into three different tubes
892 and induced with different arabinose concentrations for 2 h. Then, aliquots from all the
893 samples were taken and frozen with 60% glycerol 1:1. The remaining culture
894 transformed with pBLADE(FP6*)-mCherry was diluted to $OD_{600} = 0.15$ with pre-
895 warmed TB and transferred to a dark tube, while the cultures transformed with
896 pBAD33-mCherry were centrifuged at 6000 g for 4 min and resuspended with the same
897 volume of TB. The centrifugation and resuspension steps were repeated a second time
898 to further remove the arabinose from the medium. Then, the cultures were transferred
899 to another glass tube. All the cultures were subjected to a total of three cycles.

900

901 **Measurement of the kinetics of BLADE- and AraC-mediated mCherry**
902 **expression.** Chemically competent *E. coli* MG1655 cells were transformed with
903 pBLADE(FP6*)-mCherry, pReporter_only and pBAD33-mCherry. The overnight
904 cultures were diluted to $OD_{600} = 0.1$ and let grow in the dark to $OD_{600} = 0.4$. 100 μl of
905 each sample were collected, mixed with 100 μl of a transcription and translation
906 inhibition solution (500 $\mu\text{g ml}^{-1}$ rifampicin and 50 $\mu\text{g ml}^{-1}$ doxycycline in phosphate-
907 buffered saline), incubated in the dark 90 min at 37°C with 110 rpm shaking, diluted
908 1:1 with 60% glycerol, and frozen at -80°C. Then, the culture transformed with
909 pBLADE was split into 4 tubes, of which 3 were induced with blue light of different

910 intensities and one was kept in the dark. The cultures transformed with pBAD33-
911 mCherry were split into 4 cultures, of which 3 were induced with different arabinose
912 concentrations and one was kept without arabinose. Every hour for 6 hours, 100 µl of
913 each sample was collected, mixed with 100 µl of the transcription and translation
914 inhibition solution, incubated in the dark 90 min at 37°C with 110 rpm shaking, diluted
915 1:1 with 60% glycerol, frozen at -80°C and subsequently analyzed with the flow
916 cytometer.

917

918 **Light intensity titration.** Chemically competent *E. coli* MG1655 cells were
919 transformed with pBLADE(FP6**)-mCherry and pReporter_only. The overnight
920 culture of the cells transformed with pBLADE(FP6**)-mCherry was diluted and split
921 into 5 independent cultures, each of which was induced with blue light of different
922 intensity (which was tuned adjusting the voltage in the power supply connected to the
923 LEDs) for 4 h. The overnight culture of the cells transformed with pReporter_only was
924 diluted and grown in the dark for 4 h. 200 µl of each sample were then collected, mixed
925 with 200 µl of the transcription and translation inhibition solution, incubated in the dark
926 90 min at 37 °C with 110 rpm shaking, diluted 1:1 with 60% glycerol, frozen at -80 °C
927 and subsequently analyzed with the flow cytometer.

928

929 **Bacterial photography.** Chemically competent *E. coli* MG1655 cells were
930 transformed with pBLADE(FP6*)-sfGFP. The overnight culture was diluted in LB to
931 $OD_{600} = 0.1$ and grown for approximately 6 h. A 96-well lid (12.7 x 8.5cm) was filled
932 with 30-40 ml of 1% LB-agar and let solidify. 1 ml of the culture was then mixed with
933 9 ml of 0.4% agar at 42 °C (measured with infrared thermometer TFA Dostmann
934 (Wertheim-Reicholzheim, Germany)) and plated on top of the solidified agar in the 96-

935 well lid. The plate was covered with a transparent plexiglass sheet (12.5 x 8.3 cm) with
936 the Blade Runner movie poster sticker. For the bacteriograph of Michelangelo's
937 "Creation of Adam", the same protocol was followed, but 350-400 ml of 1 % LB-agar
938 were poured in an 18 x 15 cm case, for a total photomask area of 15.5 x 9 cm. The
939 plates were then placed in a 37 °C incubator under blue light overnight. The next
940 morning, the plates were imaged with a Zeiss Axio Zoom.V16 stereo zoom microscope
941 equipped with PlanNeoFluar Z 1.0x objective, zoom 0.7x, AxioCam MR R3 camera,
942 and the 38 HE filter set (Ex BP 470/40, FT 495, Em BP 525/50; sfGFP).

943

944 **DIC and fluorescence microscopy.** 5 µl of the bacterial culture were applied to a thin
945 agarose pad composed of 1% agarose for microscopy at room temperature and of 1%
946 agarose and 0.1% LB in tethering buffer (10 mM potassium phosphate, 0.1 mM EDTA,
947 1 mM L-methionine and 10 mM sodium lactate; pH 7.0) for long-term microscopy at
948 37°C. Images were acquired on a Zeiss Axio Observer Z1/7 fluorescence microscope
949 equipped with the Colibri 7 LED light source, an Alpha Plan-Apochromat 100x/1.46
950 Oil DIC (UV) M27 objective, filter sets 38 HE (Ex BP 470/40, FT 495, Em BP 525/50;
951 sfGFP), 108 HE (Ex BP 423/44, DBS 450+538, Em DBP 467/24+598/110; MM 4-64),
952 96 HE (Ex BP 390/40, FT 420, Em BP 450/40; DAPI), 64 HE (Ex BP 587/25, FT 605,
953 Em BP 647/70; mCherry) and an Axiocam 506 Mono camera.

954 The induction of gene expression in selected cells within a population of MG1655 cells
955 transformed with pBLADE(FP6**)-sfGFP was performed on a Zeiss LSM 800
956 confocal microscope. An area of 6.4 µm² was illuminated with a 488 nm diode laser
957 (10 mW) at 0.1% intensity, with a frame average of 8, resulting in 0.36 µs of light per
958 pixel. The illumination was given in pulses of 5 min for a duration of 3h.

959

960 **FRAP.** The overnight culture was diluted in the morning in fresh TB medium to OD_{600}
961 = 0.1, and grown until it reached $OD_{600} = 0.4$. 5 μ l of the culture were then applied to
962 a thin 1% agarose pad. Cells showing foci were manually selected. The whole
963 fluorescent focus was bleached with a single 1 s pulse of a 488 nm diode laser (10 mW)
964 at 50% intensity on a Zeiss LSM 800 confocal microscope. Images in the GFP channel
965 (filter set 38 HE: Ex BP 470/40, FT 495, Em BP 525/50) were taken before,
966 immediately and 15-min post bleaching.

967

968 **Induction of *rodZ* in KC717 cells.** Strain KC717 (kind gift of KC Huang, Stanford
969 University) was grown in LB medium supplemented with 0.2% arabinose (to maintain
970 the cells rod-shaped) during the preparation of chemically competent cells and culturing
971 for DNA extraction procedures. The blue light and arabinose induction were performed
972 as described above. The recovery phase of the culture induced with arabinose was
973 performed by centrifuging the cells at 4000 g for 4 min and resuspending them with the
974 same volume of LB twice. The culture was then diluted to $OD_{600} = 0.1$. The recovery
975 phase of the culture transformed with pBLADE^{ONLY_C} (based on pBAD33) was
976 performed by dilution to $OD_{600} = 0.1$ and incubation in the dark.

977

978 **BLADE (FP6) expression and purification.** Chemically competent *E. coli* Rosetta
979 (DE3) cells carrying the pLysS plasmid were freshly transformed with pET28a-FP6
980 and cultivated overnight in LB medium supplemented with 50 μ g ml⁻¹ kanamycin. A
981 daily culture was grown in LB medium with kanamycin at 37 °C until $OD_{600} = 0.5$, after
982 which 1 mM IPTG and 5 μ M FAD were added, and the culture was grown for 16 h at
983 18 °C under constant blue light. Cells were collected by centrifugation and the pellet
984 was re-suspended in 30 ml of lysis buffer (50 mM potassium phosphate pH 8.0, 300

985 mM NaCl and 10 mM imidazole pH 8.0) supplemented with a cOmplete™ protease
986 inhibitor cocktail tablet (Roche). Cell lysis was performed by sonication and the lysate
987 was centrifuged at 20,000 rpm for 20 min at 4 °C. The supernatant was then incubated
988 with 1 ml of HisPur™ Ni-NTA Resin (Thermo Scientific) for 2 h at 4 °C. Protein
989 purification was performed by the gravity flow method. The bound proteins were
990 washed twice with 5 ml of wash buffer (lysis buffer + 10 % glycerol + 20 mM
991 imidazole) and finally eluted with 1.5 ml of elution buffer (50 mM potassium phosphate
992 pH 7.5, 300 mM NaCl, 500 mM imidazole pH 8.0 and 10 % glycerol). The elution
993 buffer was replaced with a storage buffer (20 mM HEPES-NaOH pH 7.5, 150 mM
994 NaCl and 10 % glycerol) using an Amicon® Ultra-4 regenerated cellulose NMWL 10
995 kDa centrifugal filter unit (Merck). The protein was then stored as 50 µl aliquots at -80
996 °C.

997

998 **Spectroscopy.** The absorption spectrum of the FAD cofactor bound to VVD within
999 BLADE (FP6) was measured exciting the sample in the 300-600 nm range using a
1000 Multiskan GO (Thermo Scientific) plate reader. The protein sample was incubated 4
1001 days at 4 °C in the dark in a buffer solution (25 mM HEPES, 150 mM NaCl, 10%
1002 glycerol, 0.1% EDTA; pH 7.5) and then diluted to 0.5 mg ml⁻¹. The same sample was
1003 then illuminated with blue light (455 nm; 50 W/m²) for 5 min at room temperature and
1004 the absorption spectrum in the lit state was measured. The absorption spectrum of the
1005 blank (only medium) was subtracted from the dark and lit state spectra.

1006

1007 **SEC.** Purified BLADE (FP6) was thawed and stored in complete darkness at 4 °C for
1008 6 days. The sample (1 ml of protein with a concentration of 0.5 mg ml⁻¹) was loaded
1009 onto a Superdex™ 75 Increase 10/300 GL (GE Healthcare Lifesciences) column at 4

1010 °C. The running buffer consisted of 20 mM HEPES-NaOH pH 7.5, 150 mM NaCl and
1011 10 % glycerol, and the flow rate was adjusted to 0.25 ml min⁻¹. For the lit sample, the
1012 protein was incubated under constant blue light (455 nm; 50 W/m²) for 30 min at 4°C,
1013 prior to injection. During the run, the column was either illuminated with constant blue
1014 light (460 nm; 8 W/m², lit sample) or kept in complete darkness (dark sample). Bovine
1015 serum albumin (BSA) and carbonic anhydrase (CA) were used as molecular markers at
1016 a concentration of 0.5 mg ml⁻¹ each.

1017

1018 **Quantification of cell length, width and roundness.** The cell length and width were
1019 calculated by first staining the cell with the membrane dye MM 4-64 (AAT Bioquest
1020 Sunnyvale, CA) to visualize the cell contour, and then manually measuring the long
1021 and short axes of the cell, respectively, using the straight-line ‘Selection’ tool of Fiji (V
1022 2.0.0). The histograms were generated in Excel (2019) by the Analysis ToolPak’s
1023 Histogram option. The roundness was calculated in Fiji using the formula $\frac{4 \cdot Area}{\pi \cdot [Major\ axis]^2}$
1024 after setting manually the oval ‘Selection’ tool on each cell.

1025

1026 **Mathematical modelling.** The LacI IPTG dose-response was fitted to a Hill equation
1027 of the following form:

1028

$$f(x) = r_{\max} \frac{x^n}{k_m + x^n}$$

1029 where $f(x)$ describes the gene expression controlled by LacI, x represents the IPTG
1030 concentration, r_{\max} is the maximal promoter expression, k_m is IPTG’s dissociation
1031 constant for LacI, and n is the Hill coefficient for LacI. This dose-response was used
1032 subsequently to obtain IPTG concentration estimates from the fluorescence readouts of
1033 the constitutive promoters. All data were fitted using a non-linear least squares

1034 optimizer (MATLAB V R2015a 8.5.0.197613, MathWorks) with fitted parameter
1035 values $r_{\max} = 21352$, $k_m = 62$, $n = 1.7$.

1036

1037 **Growth on L-arabinose.** Cultures were inoculated from glycerol stocks and grown for
1038 24 h in M9 medium supplemented with 0.001% thiamine, 0.00006% ferric citrate, 0.1
1039 mM calcium chloride, 1 mM magnesium sulfate. 2x M9 medium supplemented with
1040 0.001% casamino acids, 0.002% thiamine, 0.00012% ferric citrate, 0.2 mM calcium
1041 chloride, 2 mM magnesium sulfate was prepared. Either 2 ml of 20% L-arabinose in
1042 H₂O or 2 ml of H₂O was added to 8 mL medium to create medium containing 4% L-
1043 arabinose or no L-arabinose medium. IPTG was serially diluted starting with 4 mM
1044 IPTG using the 2x M9 medium. 100 μ l of preculture (OD₆₀₀ approx. 0.2) was used for
1045 inoculation of 10 ml main culture containing un-supplemented M9 medium. 100 μ l
1046 inoculated medium was mixed with 100 μ l 2x M9 medium and the respective
1047 concentration of IPTG and L-arabinose directly in the microplates. 200 μ l culture was
1048 incubated per well of a clear-bottom 96-well plates (Cell Culture Microplates 96 Well
1049 μ Clear® CELLSTAR®, Greiner Bio-One GmbH, Product #: 655090), which were
1050 sealed with peelable foil (Sealing foil, clear peelable for PlateLoc, No. 16985-001,
1051 Agilent) to prevent liquid evaporation and guarantee sterility, as well as a plastic lid
1052 (Greiner Bio-One GmbH, Product #: 656171). For pre- and main cultures, 34 μ g ml⁻¹
1053 chloramphenicol was used. Cultures were grown at 37°C with shaking at 300 rpm as
1054 described in the Methods section on bacterial growth. Optical density of the samples
1055 was measured at 600 nm and 9 nm bandwidth, using a Tecan infinite 200Pro and
1056 Firmware V 3.40 in absorbance mode, with 2 x 2 reads per well (Square filled), 2300
1057 μ m (border), 10 flashes and 200 msec settle time. The mean of the four measurement
1058 was used. All growth experiments, except for the light titration, were performed in

1059 triplicates, and on two different days. For the light titration experiments, the same initial
1060 culture was aliquoted and incubated under the respective light conditions.

1061

1062 **Data availability**

1063 pBLADE(FP6*)-mCherry, pBLADE(FP6**)~mCherry, pBLADE^{ONLY_A} and
1064 pBLADE^{ONLY_C} have been deposited at Addgene (IDs: 168048, 168049, 168050 and
1065 168051, respectively). All other plasmids constructed in this study are additionally
1066 available from the corresponding authors upon reasonable request. Source data are
1067 provided with this paper.

1068

1069

1070

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