Light-responsive control of bacterial gene expression: Precise triggering of the *lac* promoter activity using photocaged IPTG

Dennis Binder,*[a] Alexander Grünberger,*[b] Anita Loeschcke,[a] Christopher Probst,[b] Claus Bier,[c] Jörg Pietruszka,[b,c] Wolfgang Wiechert, [b] Dietrich Kohlheyer,[b] Karl-Erich Jaeger,[#a,b] and Thomas Drepper[#a]

[a] Institute of Molecular Enzyme Technology, Heinrich-Heine-University Dusseldorf, Research Center Jülich, Germany.

[b] Institute of Bio- and Geosciences, IBG-1: Biotechnology, Systems Biotechnology, Research Center Jülich, Germany

[c] Institute for Bioorganic Chemistry, Heinrich-Heine-University Düsseldorf, Research Center Jülich, Germany

[#] e-mail: k.-e.jaeger@fz-juelich.de or t.drepper@fz-juelich.de

* These authors contributed equally to this work.

The Supplementary material includes:

Supplementary table

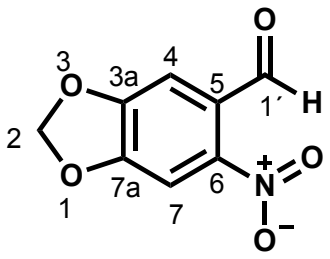
Tab. S1 Strains and Plasmids applied in this work

Strains and plasmids	Relevant characteristics and genotype	References
***E. coli* strains**		
DH5α	*F⁻ Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rk^-, mk^+) phoA supE44λ⁻ thi-1 gyrA96 relA1*	Hanahan 1983
BL21(DE3)	*F⁻ ompT gal dcm lon $hsdS_B(r_B^- m_B^-)$λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])*	Studier & Moffatt 1986
Tuner(DE3)	*F⁻ ompT gal dcm lon $hsdS_B$ (r_B^- m_B^-) lacY1(DE3)*	Novagen
Plasmids		
pRhotHi-2	pBBR1-MCS-derivative, P_{T7}-lacO-MCS, Km^R, Cm^R	Katzke *et al*. 2010
pRhotHi-2-EYFP	pBBR1-MCS-derivative, P_{T7}-lacO-MCS, Km^R, Cm^R, EYFP	Katzke *et al*. 2010
pBBR22b	pBBR1-MCS-derivative, P_{T7}-lacO-MCS, lacI, Cm^R	Rosenau & Jaeger, 2004
pBSL15	Km^R (*aphII*)	Alexeyev, 1985
pRhotHi-2-LacI	pBBR1-MCS-derivative, P_{T7}-lacO-MCS, Km^R, Cm^R, pBBR22b-lacI	this work
pRhotHi-2-LacI-EYFP	pBBR1-MCS-derivative, P_{T7}-lacO-MCS, Km^R, Cm^R, pBBR22b-lacI, EYFP	this work

Supplementary methods

Synthesis and analysis of 6-nitropiperonal [1]

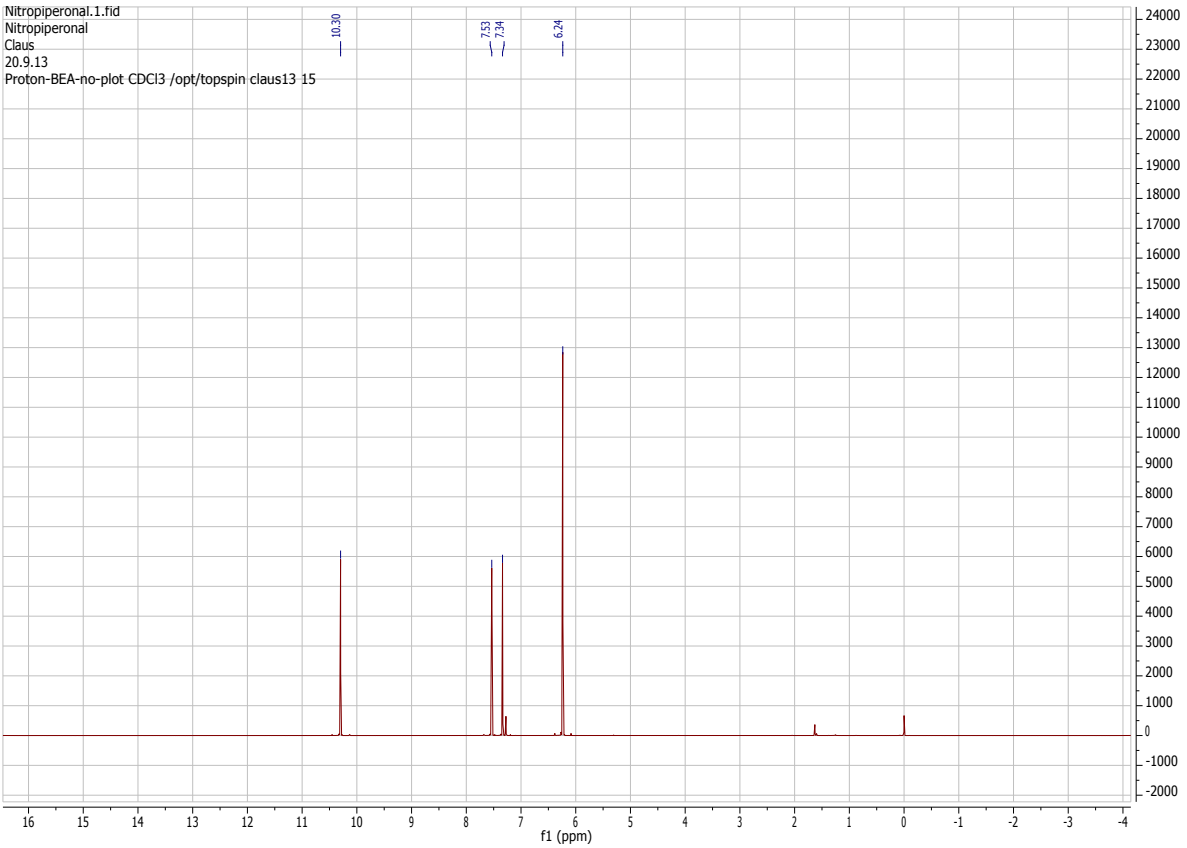
Under absence of light, piperonal (10.0 g, 66.6mmol) was added to 70% nitric acid (56 mL) at 0 °C. During the addition the temperature should not rise above 0 °C. Afterwards the reaction mixture was stirred for 4h. The mixture was poured on ice. The precipitation was collected and washed with ice water. The product was recrystallized from ethanol to yield a yellow solid (12.35 g, 95%).

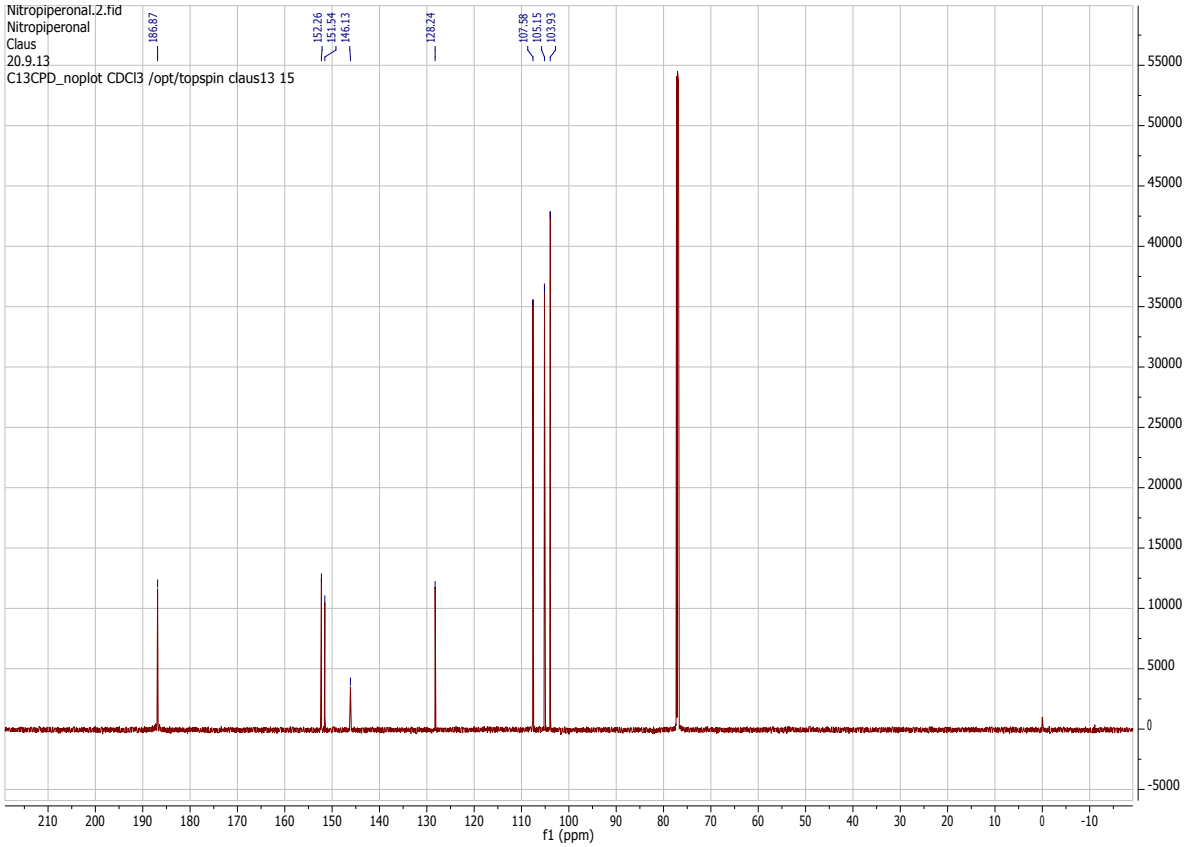


NMR data:
δ_H (600 MHz; $CDCl_3$) 6.24 (2 H, s, 2´-H), 7.34 (1 H, s, 7-H), 7.53 (1 H, s, 4-H), 10.30 (1 H, s, 1´-H).

δ_C (151 MHz; $CDCl_3$) 103.93 (2-C), 105.15 (7-C), 107.58 (4-C), 128.24 (5-C), 146.13 (6-C), 151.54 (3a-C/ *7a-C*), 152.26 (7a-C/ 3a-C), 186.87 (1´-C).

NMR spectra of 6-nitropiperonal:





IR absorptions:

ν_{max} /cm^{-1} 3096, 3056, 2927, 1679, 1596, 1511, 1486, 1418, 1394, 1367, 1328, 1273, 1225, 1170, 1127, 1019, 922, 887, 880, 814, 790, 753, 725, 688.

Melting point:

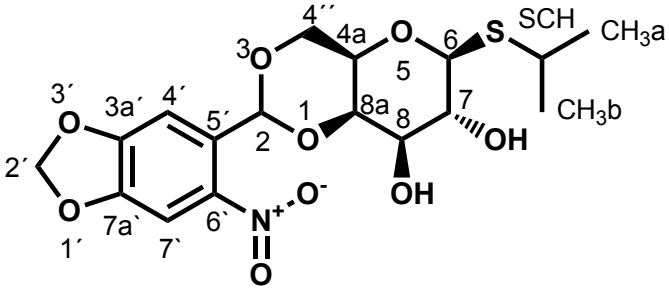
95.6°C

UV-Vis absorptions:

λ_{max} ($CHCl_3$)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 2812), 308 (3125), 348 (18438).

Analytical data of NP-photocaged IPTG [2]

(4aR,6S,7R,8R,8aR)-6-(Isopropylthio)-2-(6-nitrobenzo[d][1,3]dioxol-5-yl)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diol

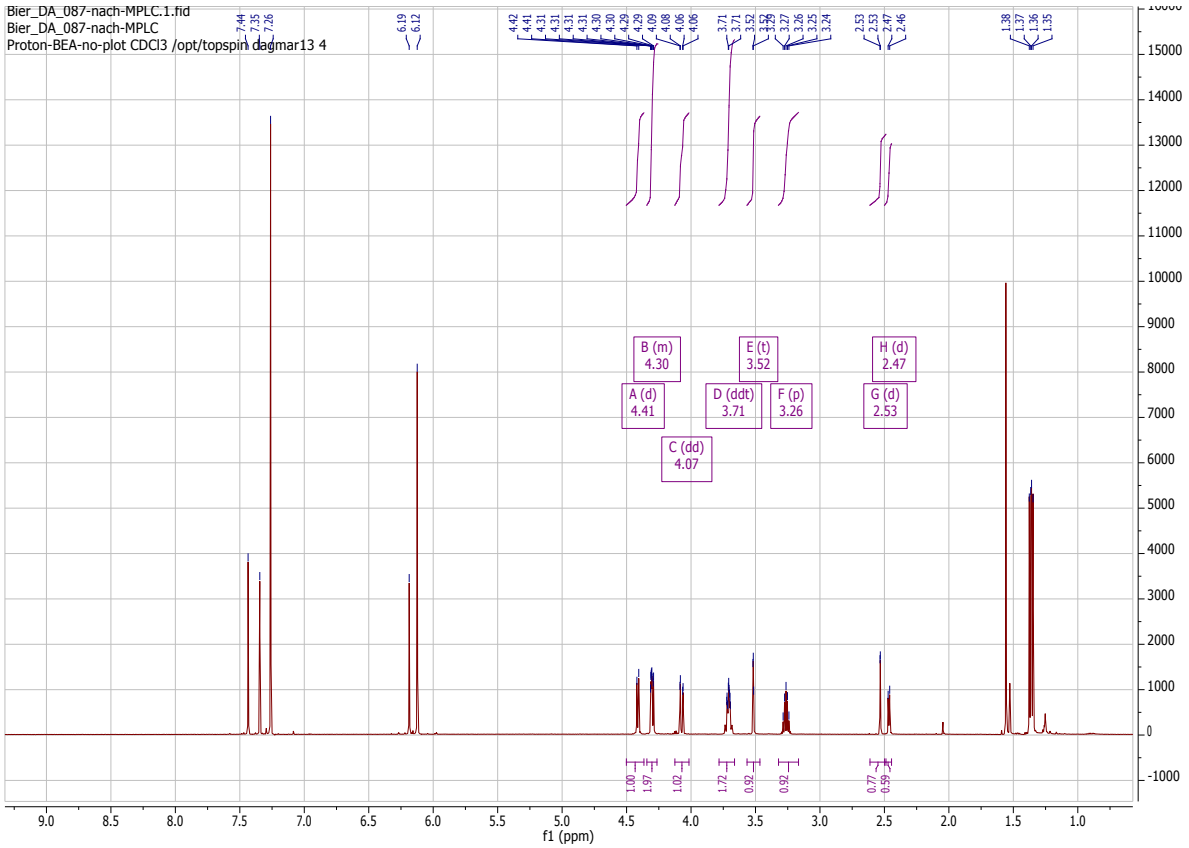


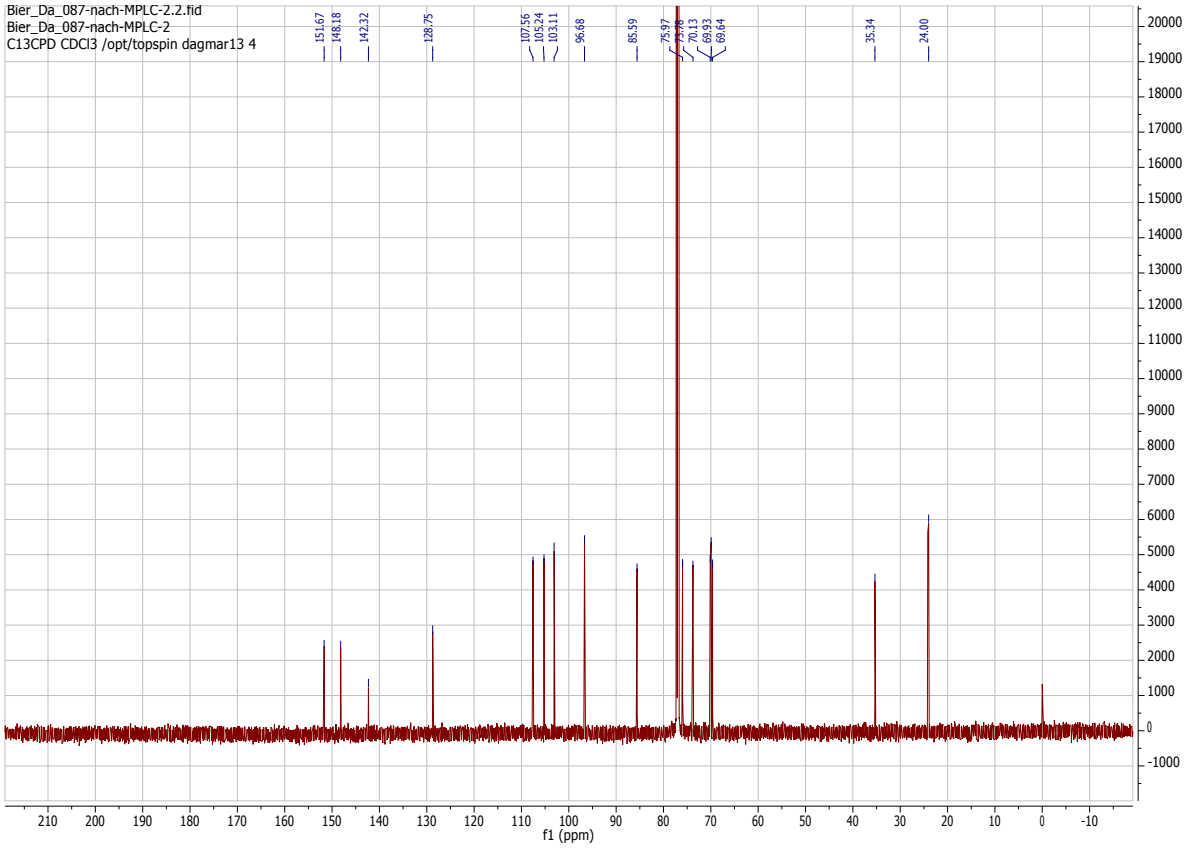
NMR data:

δ_H (600 MHz; $CDCl_3$) 1.35 (3 H, d, $^3J_{CH3a/b,\ SCH}$= 6.8 Hz, -CH_3a/b) 1.37 (3 H, d, $^3J_{CH3a/b,\ SCH}$= 6.8 Hz, -CH_3a/b), 2.54 (1 H, d, $^4J_{8,\ 7}$ = 7.4 Hz, 8-OH), 2.59 (1 H, d, $^4J_{7,\ 8}$ = 1.4 Hz, 7-OH), 3.26 (1 H, septet, $^3J_{SCH,\ CH3a/b}$ = 6.70 Hz, -SCH), 3.52 (1 H, s, 8a-CH), 3.71 (2 H, m, 7-CH, 8-CH), 4.07 (1 H, dd, $^2J_{4''a,\ 4''b}$ = 12.7 Hz, $^3J_{4''b,\ 4a}$ = 1.95 Hz, 4´´b-CH_2), 4.30 (2 H, m, 8a-CH,4´´a-CH_2), 4.41 (1 H, d,$^3J_{6,7}$ = 9.1 Hz, 6 –CH), 6.12 (2 H, s, 2´-CH_2), 6.18 (1 H, s, 2-CH), 7.35 (1 H, s, 4´-CH), 7.43 (1 H, s, 7´-CH).

δ_C (151 MHz; $CDCl_3$) 24.00 (C- CH_3a/CH_3b), 24.18 (C- CH_3a/CH_3b), 35.34 (SCH), 69.64 (C-4´´), 69.93 (C-4a), 70.13 (C-8), 73.78 (C-7), 75.97 (C-8a), 85.59 (C-6), 96.68 (C-2), 103.11 (C-2´),105.24 (C-7´), 107.56 (C-4´), 128.75 (C-6´), 142.32 (C-5´), 148.18 (C-3a´/7a´), 151.68 (C-3a´/7a´).

NMR spectra of NP-photocaged-IPTG:





IR absorptions:

ν_{max} /cm^{-1} 3596, 3410,2967, 2916, 2866, 1639, 1615, 1522, 1506, 1486, 1431, 1408, 1363, 1342, 1268, 1240,1268, 1240, 1168, 1142, 1103,1051, 989, 964, 948, 929, 906, 888, 873,841,817, 799, 758, 727.

Melting point:

164.3°C

Mass spectrometry data:

HR-MS (ESI, positive mode):

m/z = 433.1275 (33 %, M+NH_4^+), 438.0829 (100 %, M+Na).

Optical rotation:

$[\alpha]_D^{20}$ -75.2° (c =0.2 in $CHCl_3$)

UV-Vis absorptions:

λ_{max} ($CHCl_3$)/nm 251 (ε/dm^3 mol^{-1} cm^{-1} 8333), 344 (3232).

Supplementary figures

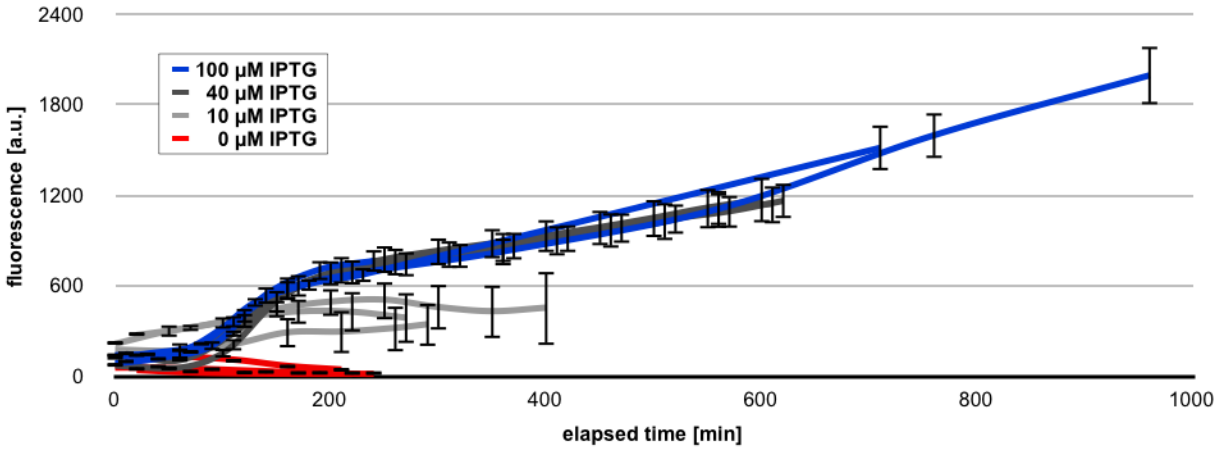


Fig. S1 Fluorescence development of *E.coli* BL21(DE3)/pRhotHi-2-EYFP microcolonies during differently supplemented microfluidic cultivation. For each concentration, fluorescence development of three independent microcolonies is plotted. Data points represent mean fluorescence values of all cells with the standard deviation as error bars. a.u.: arbitrary units

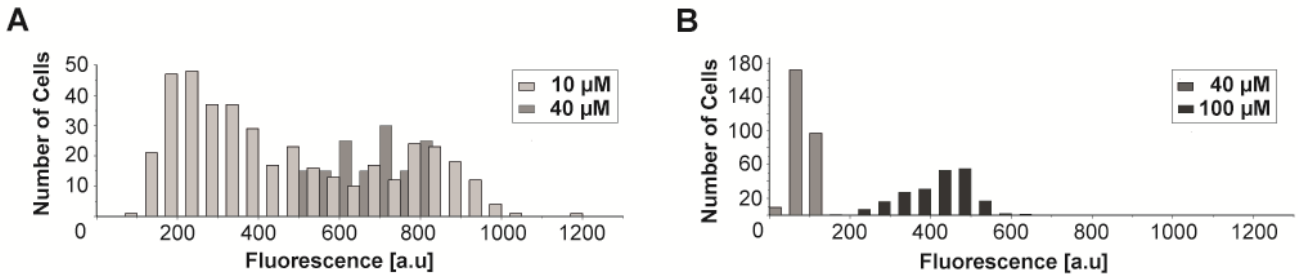


Fig. S2 Population histograms for representative microcolonies of differently induced *E. coli* BL21(DE3)/pRhotHi-2-EYFP (**A**) and Tuner(DE3)/pRhotHi-2-LacI-EYFP (**B**) populations after microfluidic cultivation for 100 min. a.u.: arbitrary units

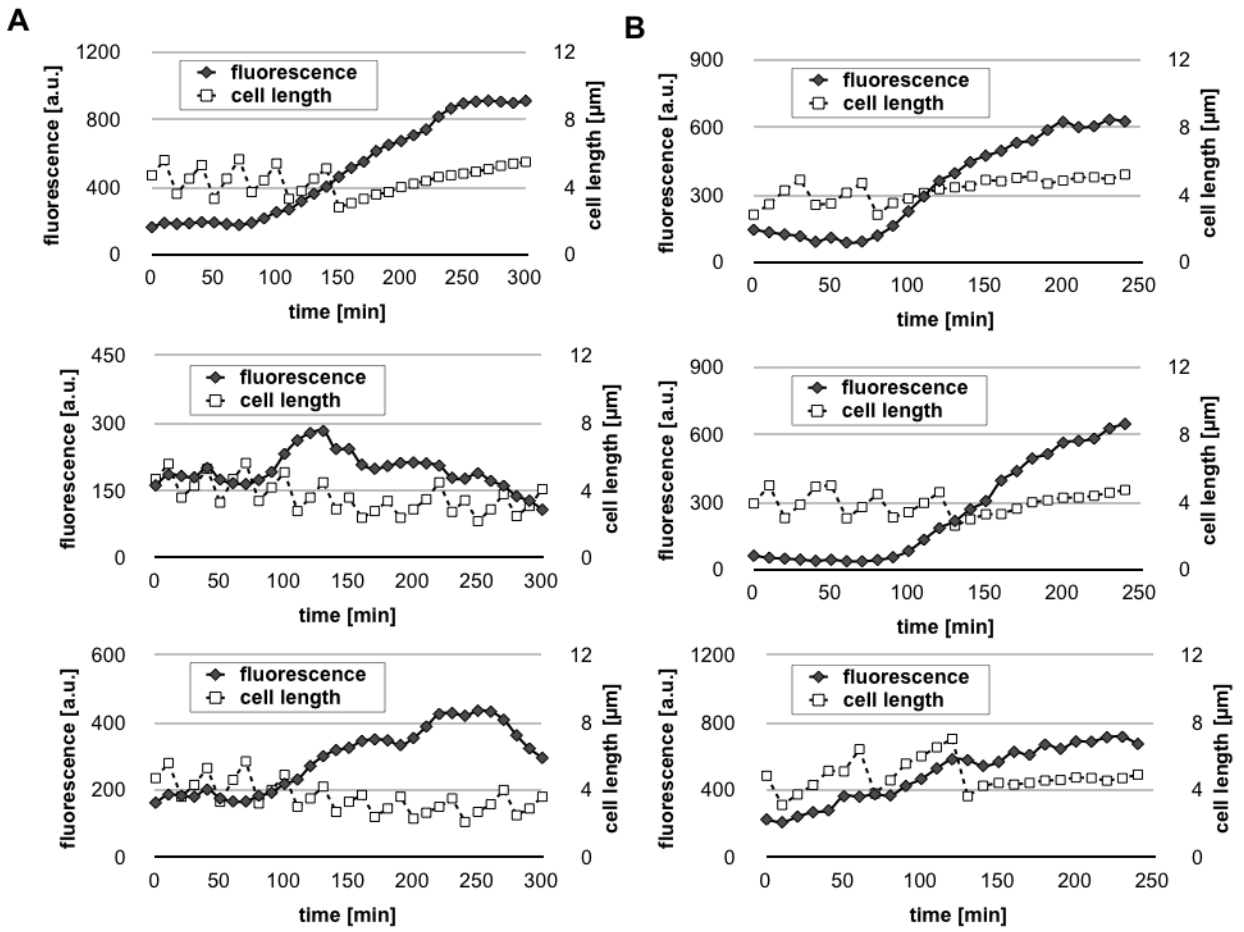


Fig. S3 Correlation between growth and fluorescence development of differently induced *E. coli* BL21(DE3)/pRhotHi-2-EYFP. Monitoring of single cell fluorescence (diamonds) and cell length (squares) as a measure for ongoing cell divisions during the induction with 10 µM (**A**) and 40 µM IPTG (**B**). a.u.: arbitrary units

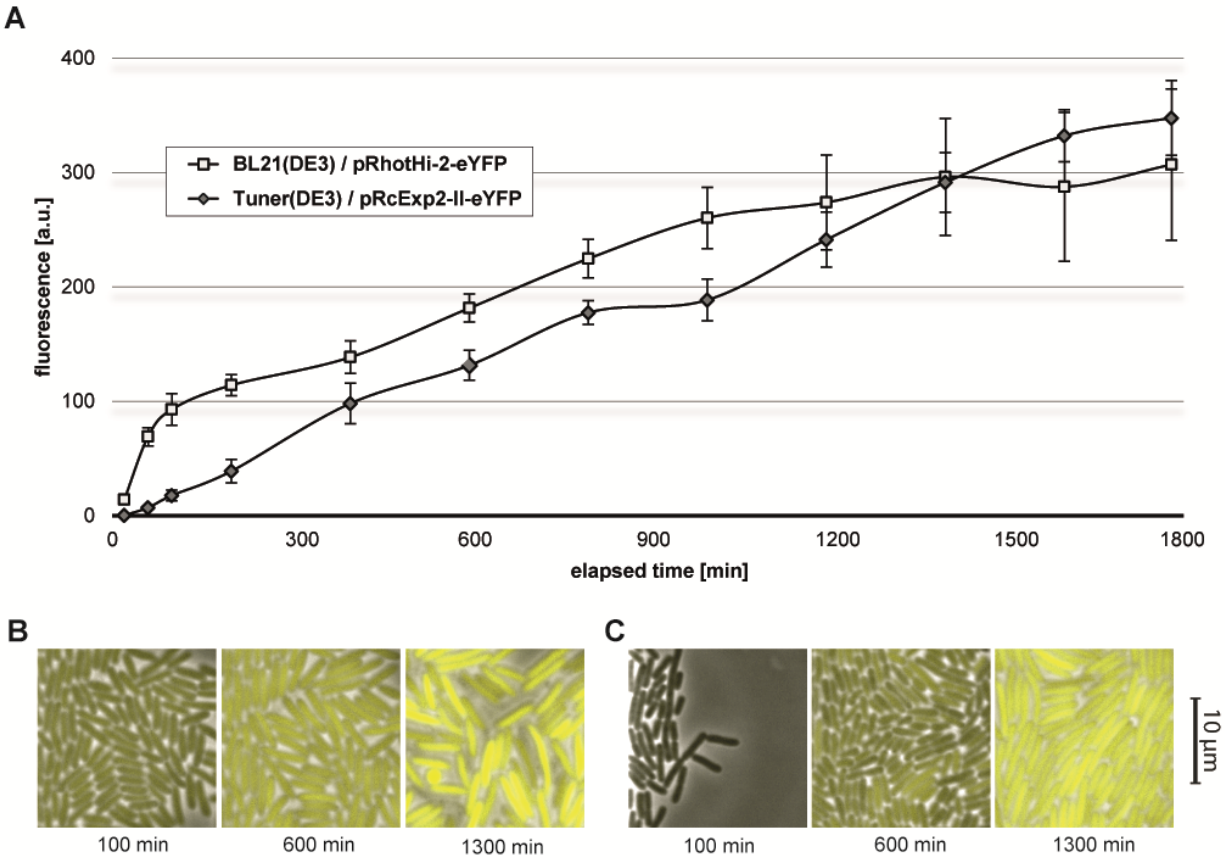


Fig. S4 **A** Long-term fluorescence development in *E. coli* BL21(DE3)/pRhotHi-2-EYFP (squares) and *E. coli* Tuner(DE3)/pRhotHi-2-LacI-EYFP (filled diamonds) during microfluidic perfusion cultivation upon full induction with 100 µM IPTG. Induction was performed after several hours of precultivation in cultivation chambers that were open at both sides [3,4] and therefore allowed for several cells to be monitored whereas others grew out of the cultivation device and were flushed away through the nutrient channel. Selected photographs of *E. coli* BL21(DE3)/pRhotHi-2-EYFP (**B**) and *E. coli* Tuner(DE3)/ pRhotHi-2-LacI-EYFP (**C**) show a delayed fluorescence in Tuner(DE3) that yields to comparable final levels at the end of the experiment. a.u.: arbitrary units

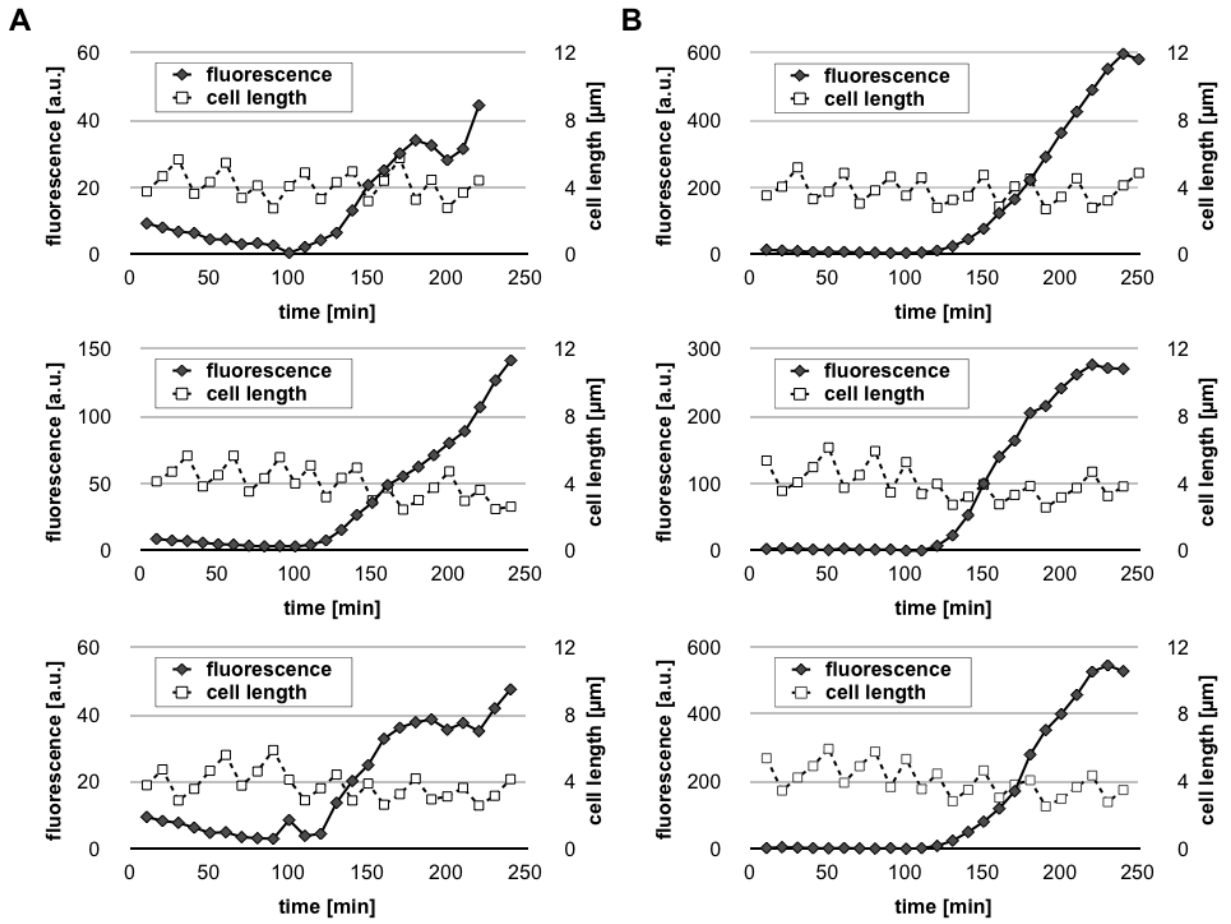


Fig. S5 Correlation between growth and fluorescence development of differently induced *E. coli* Tuner(DE3)/ pRhotHi-2-LacI-EYFP single cells. Monitoring of cell fluorescence (diamonds) and cell length (squares) as a measure for ongoing cell divisions during the induction with 40 µM (**A**) and 100 µM IPTG (**B**). a.u.: arbitrary units

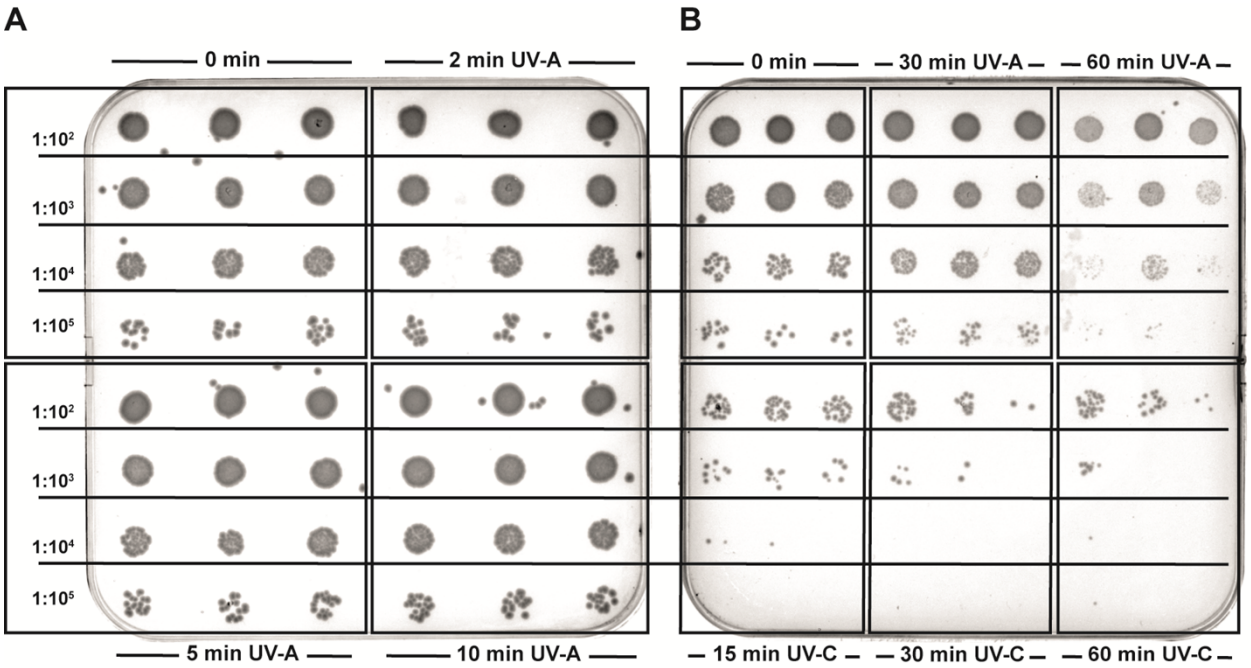


Fig. S6 Toxicity assay comparing colony forming of differently UV-exposed and serially diluted cell suspensions.
To exclude phototoxic effects caused by exposing *E. coli* cells with UV-A light, a phototoxicity assay was conducted. To analyze cell viability of differently UV-irradiated and serially diluted *E. coli* Tuner(DE3)/pRhotHi-2-LacI cells. Cultures were inoculated for 1.5 h and exposed for (**A**) 0, 2, 5 and 10 min to UV-A light and for (**B**) 0, 30 and 60 min to UV-A light (upper section) as well as 15, 30 and 60 min to UV-C light (lower section). Cultivation and exposure were performed exactly as described in the materials and methods section. UV-C exposure was realized using a low-intesity UV-C hand lamp (λ_{max}: 254 nm, 12 W, VL-6-LV from Vilber Lourmat, France). The cell cultures were diluted as indicated and 3 µl of diluted suspensions were placed on an LB-agar plate, which was cultivated over night (37°C, 50 µg/ml Kanamycin).

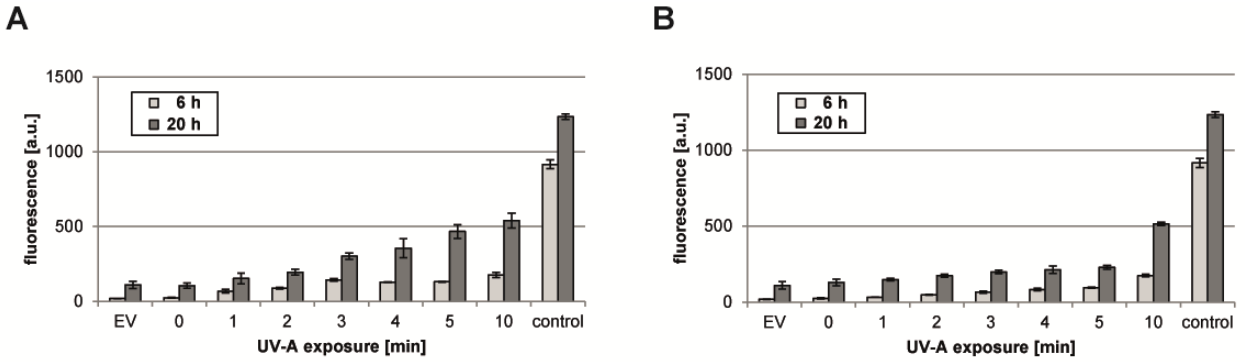


Fig. S7 Influence of NP-photocaged IPTG supplementation time on UV-A light-controlled regulation of gene expression in *E. coli* Tuner(DE3)/pRhotHi-2-LacI-EYFP.
In vivo fluorescence of *E. coli* cultures supplemented with 40 µM NP-photocaged IPTG either at the beginning of the cultivation (**A**) or directly before light induction (**B**). Gene expression was specifically induced by increasing periods of UV-A light exposure. Cultures were induced after 2 h of pre-cultivation where cells were kept in darkness. Corresponding control cultures were supplemented with 40 µM uncaged IPTG. EV: empty vector control. Values are means of triplicate measurements. Error bars indicate the respective standard deviations. a.u.: arbitrary units.

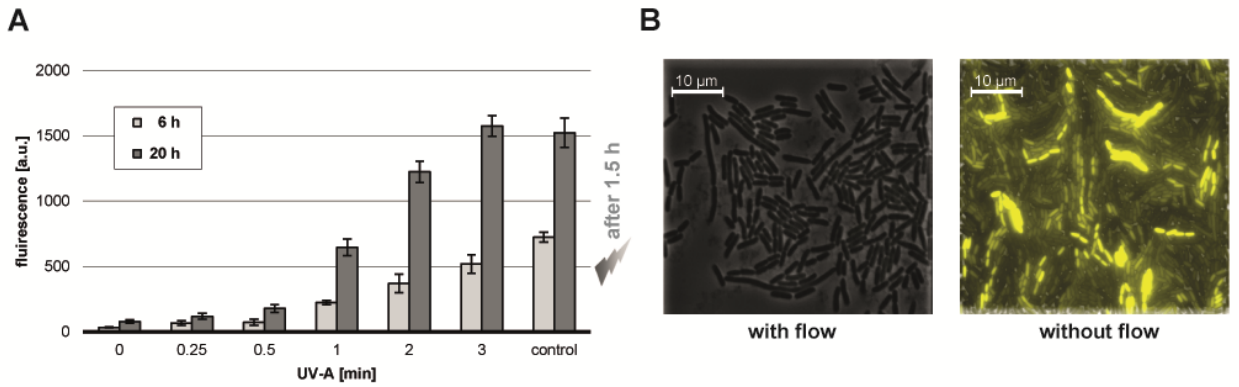


Fig. S8 UV-A light mediated regulation of gene expression in *E. coli* BL21(DE3)/pRhotHi-2-EYFP using NP-photocaged IPTG. **A** *In vivo* fluorescence of *E. coli* cultures supplemented with 40 µM NP-photocaged IPTG. Gene expression was specifically induced by increased times of UV-A light exposure. Cultures were induced after 1.5 h of pre-cultivation where cells were kept in darkness. Corresponding control cultures (control) have been supplemented with 40 µM conventional IPTG. Values are means of triplicate measurements. Error bars indicate the respective standard deviations. a.u.: arbitrary units. **B** Microscale perfusion (left) and batch (right) cultivation using pre-exposed (1 min) NP-photocaged IPTG (40 µM) supplemented LB medium after 450 min of cultivation.

Supplementary references

1. C. D. Duarte, J. L. M. Tributino, D. I. Lacerda, M. V Martins, M. S. Alexandre-Moreira, F. Dutra, E. J. H. Bechara, F. S. De-Paula, M. O. F. Goulart, J. Ferreira, J. B. Calixto, M. P. Nunes, A. L. Bertho, A. L. P. Miranda, E. J. Barreiro, and C. a M. Fraga, *Bioorganic & medicinal chemistry*, 2007, **15**, 2421–33.
2. D. D. Young and A. Deiters, *Angewandte Chemie (International ed. in English)*, 2007, **46**, 4290–2.
3. G. Ullman, M. Wallden, E. G. Marklund, A. Mahmutovic, I. Razinkov, and J. Elf, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 2013, **368**, 20120025.
4. W. Mather, O. Mondragón-Palomino, T. Danino, J. Hasty, and L. S. Tsimring, *Physical review letters*, 2010, **104**, 208101.