Electronic Supplementary Material (ESI) for Chemical Communications

Synthesis of Phycocyanobilin in Mammalian Cells

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Materials and Methods

DNA cloning. The construction of expression vectors is detailed in Supplementary Table 1.

Cell culture and transfection. Chinese hamster ovary cells (CHO-K1, ATCC CCL 61) were cultivated in HTS medium (Cell Culture Technologies) supplemented with 10% fetal bovine serum (FBS) (PAN, cat. no. P30-3602, batch no. P101003TC), and 2 mM L-glutamine (Sigma). The medium was supplemented with 100 U/ml of penicillin and 0.1 mg/ml of streptomycin (PAN). Cells were transfected, using a polyethylene-imine-based method (PEI, linear, MW: 25 kDa) (Polyscience). In brief, 1 mg/ml PEI solution in H_2O was adjusted to pH 7.0 with HCl, sterile filtered and stored at -80 °C in aliquots. 70.000 cells were seeded per well of a 24-well plate and cultivated overnight. Aliquots of 0.75 µg of DNA were diluted in 50 µl of OptiMEM (Invitrogen) and mixed with 2.5 µl of PEI solution in 50 µl of OptiMEM under vortexing (amounts scaled to one well). After 15 min incubation at room temperature, the precipitate was added to the cells. For other plate formats, the cell number and amount of reagents were scaled up according to the growth area. The culture medium was replaced 5 h after the transfection. Unless indicates, all plasmids were transfected in equal amounts (w:w) and only the bicistronic expression cassette for the PCB-biosynthesis enzymes on pKM087 was transfected in 2-fold excess (w:w).

Lentiviral particle production. For production of replication-incompetent, self-inactivating (SIN) HIV-1-based lentiviral particles, solution I (100 µl 2x HBS (280 mM NaCl, 100 mM Hepes, 1.5 mM Na_2HPO_4, pH 7.1)) was mixed with solution II (50 µl 0.5 M $CaCl_2$ and 50 µl H_2O, containing 1 µg pLTR-G, 1 µg pCD/NL-BH* and 2 µg of the desired transgene-encoding lentiviral expression vector). Following 15 min incubation at room temperature the DNA-calcium-phosphate precipitates were applied with 25 µM chloroquine to 500,000 HEK293-T cells[1] per well of a 6-well plate, seeded 24 h before transfection in Advanced D-MEM (Gibco, cat. no.: 12491-015) supplemented with 2 % (v/v) FBS, 0.01 M cholesterol, 0.01 M egg lecithin and 1x chemically defined lipid concentrate (Gibco, cat. no.: 11905-031). The HEK293-T culture medium was replaced 5 h post-transfection and lentiviral particles were produced for another 48 h prior to their collection from the supernatant by filtration through a 0.45 mm filter. Viral stocks were stored at -80 °C in aliquots.

Illumination conditions. Light intensity was adjusted to 8 µmol m^{-2} sec^{-1}, for red (660 nm) light illumination and to 80 µmol m^{-2} sec^{-1} for far-red (740 nm) light illumination. Illumination was performed by custom-made LED arrays and the light intensity was measured, using a quantum

sensor (LI-COR, prod. no.: Q45045). All cell-handling involving the red light-inducible expression systems was done under safe 522 nm light, when PCB was present in the system.

Reporter gene and biliverdin reductase assay. The reporter SEAP was quantified in the cell culture medium, using a colorimetric assay as described elsewhere[2] and biliverdin reductase activity was determined using the Biliverdin Reductase Assay Kit (Simga, cat. no.: CS1100)

Mathematical modeling. In order to calculate the PCB production rate we used the mathematical model and the experimental data which we published in [3]. To describe the experiments in this study we refined eq. (1) of the model to

$$\frac{d[PCB](t)}{dt} = GD\ k_{synth,PCB} - (k_{deg,PCB,spont} + k_{deg,PCB,ind,BVRA})\ [PCB] - k_{form,PPV}\ [PhyB\text{-}VP16]\ \frac{[PCB]}{K_{m,PCB}+[PCB]} \quad (1)$$

The PCB synthesis rate is described by the newly introduced parameter $k_{synth,PCB}$. In this study we used CHO-K1 cells with BVRA knock-down. Therefore the description of the PCB degradation was also refined. The parameter $k_{deg,PCB,spont}$ models the spontaneous degradation of PCB independent of BVRA. The parameter $k_{deg,PCB,ind,BVRA}$ describes induced degradation of PCB by BVRA. In cells with BVRA knock-down $k_{deg,ind,BVRA}$ is around 70 % smaller than in wild type cells (Fig. 2a).

The model was parameterized by performing a multi-experiment fit to the data shown in Supplementary Figure 1 and the data acquired in [3]. The fitting was done by maximizing the likelihood function, see Supplementary Data in [3] for details. The confidence interval of the estimated parameters were obtained by calculating the profile likelihood.[4]

Implementation of the experiments

The experiments from [3] were implemented in the same way except for experiment 4 where we introduced an additional offset in the mRNA observation function to describe unspecific bindings in the mRNA measurement:

$$[mRNA_{t,measured}] = \text{offset}_{mRNA} + [mRNA](t).$$

In the experiment shown in Supplementary Figure 1 of this study the cells were incubated for 72 hours in $DMEM_{complete}$. During this time we assumed a slowed cell doubling time of 36 hours ($k_{growth} = 0.0193\ h^{-1}$). After 72 hours the medium was exchanged. In the new medium $HTS_{complete}$ we assumed a cell doubling time of 14 h ($k_{growth} = 0.0495\ h^{-1}$) like in [3]. Then, increasing concentrations $\text{PCB}_{\text{input}}$ of PCB were added to the medium. This was modeled by a Dirac delta pulse which was approximated by a Gaussian function and was added to the right hand side of eq. (1):

$$[PCB_{input,rate}](\text{t}) = \text{PCB}_{\text{input}}\ \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(t-\mu)^2}{2\sigma^2}\right)$$

with $\mu = 72.04\ h$ and $\sigma = 0.03\ h$. This leads to a step like increase of the PCB concentration at $t \approx 72\ h$. To model the BVRA knock-down, the induced PCB degradation was decreased by the factor $k_{BVRA,ko}$

$$k_{deg,ind,BVRA} \rightarrow k_{BVRA,ko}\ k_{deg,ind,BVRA}.$$

According to Figure 2a we used $k_{BVRA,ko} = 0.296$. After 73 hours the system was illuminated with 660 nm light which activated the PCB-PhyB-VP16 complex (PPV). Therefore the light condition was modeled with $k_{light} = 0$ for $t < 73\ h$ and $k_{light} = 1$ for $t \geq 73\ h$. After 74 hours the light was turned off which means that no more active PCB-PhyB-VP16 (PPV) was produced. We modeled this by setting $k_{form,PPV} = 0$ for $t \geq 74\ h$. The gene dose was set to $GD = 1$ at the time point ($t = 72\ h$) of the medium exchange which corresponds to the experiments in [3]. As observation function we used

$$[SEAP_{measured}] = \mathrm{scale}_{\mathrm{pKM048}}\ N\ [SEAP].$$

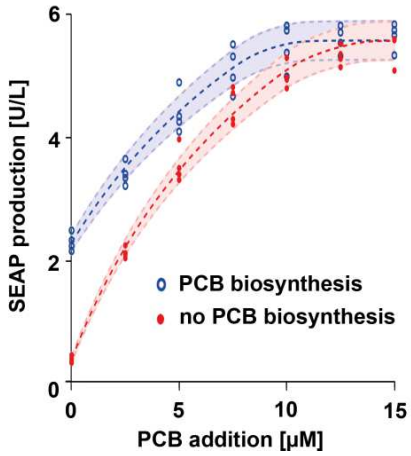
Prediction of the total PCB concentration

With the obtained parameters (Supplementary Table 3) we predicted the time course of the total PCB concentration (Fig. 3) by simulating

$$\mathrm{PCB_{total}(t)} = \mathrm{N([PCB](t) + [PPV](}t\mathrm{)).}$$

The 95% confidence interval of the prediction was calculated by propagating the uncertainties of the estimated model parameters to the predicted time course.[5]

Supplementary Figure 1



Quantification of PCB biosynthesis. Dose-response curves for PCB addition to the culture medium. CHO-K1 cells with $shRNA_{BVRA}$ were transfected for red light-inducible gene expression and for bicistronic expression of the mitochondria targeted PCB biosynthetis genes (blue) or with an empty vector (red). After incubation in the dark in $DMEM_{complete}$ for 72 h the culture medium was replaced with $HTS_{complete}$ containing increasing concentrations of PCB. After incubation in the dark for 1 h, the cells were illuminated with 660 nm light for 1 h followed by incubation in the dark for 23 h and determination of SEAP activity. The curves represent the model fit to the data, and the shaded error bands are estimated by a simple error model with a constant Gaussian error.

Supplementary Table 1.

Expression vectors and oligonucleotides designed and used in this study

Plasmid	Description	Ref. or source
pCD/NL-BH*	HIV-1-derived gag/pol-encoding helper plasmid	[6]
pKM006	Vector encoding SEAP under the control of a modified P_{Tet} harboring a 422 bp spacer between the 13-mer tetO operator and the minimal promoter ($tetO_{13}$-422bp-$P_{hCMVmin}$-SEAP-pA)	[3]
pKM022	Bicistronic vector encoding PhyB(1-650)-VP16-NLS and TetR-PIF6(1-100)-HA under control of P_{SV40} (P_{SV40}-PhyB(1-650)-VP16-NLS-$IRES_{PV}$-TetR-PIF6(1-100)-HA-pA)	[3]
pKM048	Lentiviral vector encoding $shRNA_{BVRA}$ under control of P_{hU6} (P_{hU6}- $shRNA_{BVRA}$-pA) Oligos oKM026 (5'-ccggGCCAAATGTAGGAGTCAATAActcgagTTATTGACTCCTACATTTGGCtttttg-3') and oKM027 (5'-aattcaaaaaGCCAAATGTAGGAGTCAATAActcgagTTATTGACTCCTACATTTGGC-3') were annealed and cloned (*Age*I/*Eco*RI) into pLKO.1-puro.	This work
pKM071	Plasmid encoding PcyA under control of $P_{EF1\alpha}$ ($P_{EF1\alpha}$-tePcyA-pA) Codon-optimized PcyA was chemically synthesized (Supplementary Table 2), digested (*Kpn*I/*Eco*RV) and ligated (*Kpn*I/*Eco*RV) into pWW029.	This work
pKM072	Plasmid encoding HO1 under control of $P_{EF1\alpha}$ ($P_{EF1\alpha}$-teHO1-pA) Codon-optimized HO1 was chemically synthesized (Supplementary Table S2), digested (*Kpn*I/*Eco*RV) and ligated (*Kpn*I/*Eco*RV) into pWW029.	This work
pKM074	Plasmid encoding MTS-PcyA under control of $P_{EF1\alpha}$ ($P_{EF1\alpha}$-MTS-PcyA-pA) Oligos oKM076 (5'-cGCCACCATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGCT CGGCCCGGCGGCTCCCAGTGCCGCGCGCCAAGATCCATTCGTTGggtac-3') and oKM077 (5'-cCAACGAATGGATCTTGGCGCGCGGCACTGGGAGCCGCCGGGCCGAGCCTGTCAAGCCCCGCAG CAGCAGCGGCGTCAGGACGGACATGGTGGCggtac-3') were annealed and ligated (*Kpn*I) into pKM071.	This work
pKM075	Plasmid encoding MTS-HO1 under control of $P_{EF1\alpha}$ ($P_{EF1\alpha}$-MTS-HO1-pA) Oligos oKM076 (5'-cGCCACCATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTTGACAGGCT CGGCCCGGCGGCTCCCAGTGCCGCGCGCCAAGATCCATTCGTTGggtac-3') and oKM077 (5'-cCAACGAATGGATCTTGGCGCGCGGCACTGGGAGCCGCCGGGCCGAGCCTGTCAAGCCCCGCAG CAGCAGCGGCGTCAGGACGGACATGGTGGCggtac-3') were annealed and ligated (*Kpn*I) into pKM072.	This work
pKM087	Bicistronic vector encoding MTS-PcyA and MTS-HO1 under control of P_{SV40} (P_{SV40}-MTS-PcyA-$IRES_{PV}$-MTS-HO1-pA) $IRES_{PV}$ was amplified from pKM022 using oligos oKM086 (5'-gttcaggcggccgcAAGCTTAAAACAGCTCTGGGGTTG-3') and oKM087 (5'-gttcagtctagagaattcCAATTCGCTTTATGATAACAATCTGTG-3') and digested (*Not*I/*Eco*RI), while MTS-teHO1 was amplified from pKM075 using oligos oKM088 (5'-gttcaggaattcGCCACCATGTCCGTCCTG-3') and oKM089 (5'-gttcagtctagaCACTGTGCTGGATATCAAGCTTTC-3') and digested (*Eco*RI/*Xba*I). Finally, both fragments were ligated (*Not*I/*Xba*I) into pKM074.	This work
pLKO.1-puro	Empty lentiviral vector for shRNA expression (P_{hU6}-stuffer-pA)	[7]
pLKO.1-scramble shRNA	Lentiviral vector coding for a random shRNA under control of P_{hU6} (P_{hU6}-$shRNA_{scramble}$-pA)	[8]
pLTR-G	Vector encoding VSV-G under control of 5'LTR (5'LTR-VSV-G-pA)	[9]
pMK216	Vector encoding a P_{SV40}-driven PhyB(1-908)-VP16 expression unit (P_{SV40}-PhyB(1-908)-VP16-pA)	[3]
pRSet	P_{T7}-driven bacterial expression vector	Novagen
pSAM200	Constitutive TetR-VP16 expression vector (P_{SV40}-TetR-VP16-pA)	[10]
pWW029	Vector encoding the erythromycin repressor protein E under control of $P_{EF1\alpha}$ ($P_{EF1\alpha}$-E-pA)	[11]

HA, human influenza hemagglutinin derived epitope tag; BLVRA, biliverdin reductase A; HO1, heme oxygenase 1 from *Thermosynechococcus elongatus* BP-1; $IRES_{PV}$, polioviral internal ribosome entry site; mEGFP, monomeric version of enhanced green fluorescent protein; MTS, mitochondrial

targeting signal; mYFP, monomeric version of yellow fluorescent protein; NLS, nuclear localization signal from simian virus 40 large T antigen; pA, polyadenylation signal; PcyA, PCB:ferredoxin oxidoreductase from *Thermosynechococcus elongatus* BP-1; $P_{EF1\alpha}$, human elongation factor 1α promoter; P_{hCMV}, human cytomegalovirus immediate early promoter; $P_{hCMVmin}$, minimal human cytomegalovirus immediate early promoter; P_{hU6}, human U6 promoter; PhyB, Phytochrome B; PhyB(1-650), N-terminus of Phytochrome B with amino acids 1-650; PhyB(1-908), N-terminus of Phytochrome B with amino acids 1-908; PIF6, Phytochrome-interacting-factor 6; PIF6(1-100), N-terminus of Phytochrome-interacting-factor 6 with amino acids 1-100; P_{SV40}, simian virus 40 early promoter; $P_{\Delta SV40}$, truncated simian virus 40 early promoter; P_{T5lac}, chimeric promoter for T5 RNA polymerase with the lac operator; P_{Tet}, tetracycline-responsive promoter; RBS, ribosome binding site; SEAP, human placental secreted alkaline phosphatase; shRNA, small hairpin RNA; tetO, operator sequence binding TetR; TetR, tetracycline repressor protein; VP16, *Herpes simplex* virus-derived transactivation domain; VSV-G, vesicular stomatitis virus protein G.

Uppercase in oligos, annealing sequence; underlined sequence, restriction site.

Supplementary Table 2.

Nucleotide sequences of chemically synthesized DNA.

Designation	Nucleotide Sequence
HO1	5′-...gcggccgcggtaccgccaccATGACAACATCTCTGGCCACCAAGCTGAGAGAGGGCACCAAGAAAGCCCACAC CATGGCCGAGAACGTGGGCTTCGTGCGGTGCTTCCTGAAGGGAACCGTGGAAAAGTCCTCCTACCGGAAGCTGGTGGCC TCCCTGTACCACGTGTACTCCGCCATGGAACAGGAAATGGAACGGCTGAAGGACCACCCCATCGTGGGCAAGATCTACT TCCCCGAGCTGAACCGGAAGTCCTCCCTGGAACGGGACCTGACCTACTACTTCGGCTCCAACTGGCGGGAAGAGATCCC CCCATCTCCTGCCACCCAGGCCTACGTGGCCAGAATCCACGAGGTGGCCAATACCGCCCCTGAGCTGCTGGTGGCTCAC TCCTACACCAGATACCTGGGCGACCTGTCTGGCGGCCAGATTCTGAAGGGCATTGCCGAGCGGGCCATGAACCTGCAGG ATGGCGAGGGCACCGCCTTCTACAGATTCGAGTCCATCTCCGACGAGAAGGCCTTTAAGCAGCTGTACCGGCAGCGGCT GGACGAGCTGCCTGTGGATGAGGCCACCGCCGACAGAATCGTGGACGAGGCCAATGCCGCCTTCGGCATGAACATGAAG ATCTTTCAGGAACTGGAAGGCAACCTGATCCGGGCCATCGGCCAGCTGCTGTTCAACACCCTGACCCGGCGGAAGCAGA GAGGCTCTACCGAGCTGGCCACCGCTGACTGAaagcttgatatc...-3′
PcyA	5′-...gcggccgcggtaccgccaccATGTCTCTGAGACAGCACCAGCACCCCCTGATCCAGAGACTGGCCGACAGAAT CGAGGCCATCTGGCAGGCCTTTTTCCCCCTGGCCCCTTACGCCCTGCCTGAGGATCTGGGCTACGTGGAAGGCAAGCTG GAAGGCGAGCGGCTGACCATCGAGAACCACTGCTACCAGGCCCCTCCATTCCGGAAGCTGCACCTGGAACTGGCCAGAG TGGGAGAGTCCCTGGACATCCTGCACTGCGTGATGTTCCCCGAGCCCAGATACGACCTGCCCATGTTCGGCTGCGACCT CGTGGGAGGCAGAGGCCAGATCTCTGCCGCCATCGTGGATCTGTCTCCCGTGACCGGACAGCTGCCTGCCGCTTACACC TGTGCCCTGAATGCCCTGCCCAAGCTGACCTTCCGGCAGCCCAGAGAACTGCCTCCTTGGGGCCACATCTTCAGCCCTT TCTGCATCTTCATCCGGCCCCAGGGCGAGGCCGAGGAACAGCAGTTCCTGGATCGGATCGGCGAGTACCTGACCCTGCA CTGTCAGCTGTCCCAGCAGGCCGTGCCTACCGATCATCCACAGGCTGTGATCGCCGGCCAGAGACAGTACTGCCAACAG CAGCAGCAGAACGACAAGACCAGACGGGTGCTGGAAAAGGCCTTCGGCGTGCCATGGGCCGAGCGGTATATGACCACCG TGCTGTTCGACGTGCCCCCCGTGTGAaagcttgatatc...-3′

Upper case teHO1/ tePcyA, underlined sequences in 5′ -> 3′ direction, restriction sites *Not*I, *Kpn*I, *Hin*dIII, *EcoRV*.

Supplementary Table 3.

Fitted parameters obtained by a maximum likelihood estimation.

Parameter	$log10(\theta_{opt,i})$	$log10(\sigma^-)$	$log10(\sigma^+)$	Unit
$K_{m,PCB}$	0.000	-Inf	0.000[1]	μM
$K_{m,tc}$	0.711	0.586	0.846	μM
$PhyB$-VP16$_{total_0}$	0.990	0.947	1.038	μM
$k_{synth,PCB}$	-0.575	-0.628	-0.523	$\mu M \cdot h^{-1}$
$k_{basal,mRNA}$	-1.580	-2.007	-1.378	$[mRNA](0) \cdot h^{-1}$
$k_{deg,mRNA}$	-0.549	-0.618	-0.478	h^{-1}
$k_{deg,PCB,spont}$	-0.174	-0.246	-0.108	h^{-1}
$k_{deg,PCB,ind,BVRA}$	-1.084	-Inf	-0.685	h^{-1}
$k_{deg,PPV}$	-0.823	-0.869	-0.777	h^{-1}
$k_{deg,VEGF}$	-1.024	-1.421	-0.659	h^{-1}
k_{out}	-0.966	-1.411	-0.643	h^{-1}
k_{tc}	0.512	0.413	0.613	$[mRNA](0) \cdot h^{-1}$
$k_{tl,P_{pre}}$	-0.248	-0.372	-0.045	$\mathrm{ng/ml} \cdot [mRNA](0)^{-1} \cdot h^{-1})$
$k_{tl,VEGF}$	-0.778	-0.932	-0.682	h^{-1}
$k_{tl,SEAP}$	-1.980	-2.228	-1.795	$U/l \cdot (ng/ml)^{-1} \cdot h^{-1}$
$\mathrm{offset}_{\mathrm{stability,PCB}}$	-0.242	-0.260	-0.226	1
$\mathrm{offset}_{\mathrm{mRNA}}$	-0.262	-0.414	-0.153	$[mRNA](0)$
scale_{pMK048}	-0.171	-0.194	-0.147	1
$\mathrm{scale}_{\mathrm{stability,PCB}}$	-1.542	-1.579	-1.504	μM^{-1}
$\mathrm{scale}_{\mathrm{stability,PPV}}$	-0.044	-0.070	-0.018	1
$init_{P_{pre}}$	0.000[2]			ng/ml
$init_{VEGF}$	-1.281	-Inf	-0.941	ng/ml
$init_{PPV,Exp1}$	-0.367	-0.428	-0.310	μM
$\sigma_{0,Exp1}$	-0.755	-Inf	-0.563	U/l
$\sigma_{rel,Ex1}$	-1.610	-Inf	-1.121	1
$\sigma_{0,Exp2}$	-0.366	-0.484	-0.228	U/l
$\sigma_{rel,Exp2}$	-8.000	-Inf	-1.250	1
$\sigma_{0,Exp3}$	-1.915	-Inf	-1.613	1
$\sigma_{rel,Exp3}$	-8.000	-Inf	-1.468	μM^{-1}
$\sigma_{0,Exp4}$	-0.453	-0.565	-0.329	$[mRNA](0)$
$\sigma_{rel,Exp4}$	-0.489	-0.630	-0.338	1

[1] Upper bound of this parameter was set to 1 μM
[2] Initial concentration of P_{pre} was fixed to 1, because scale of P_{pre} is not accessible

$\sigma_{0,Exp5}$	-1.407	-1.664	-1.103	ng/ml
$\sigma_{rel,Exp5}$	-1.104	-1.181	-1.019	1
$\sigma_{0,pKM048}$	-1.330	-1.659	-0.899	U/l
$\sigma_{rel,pKM048}$	-1.246	-1.350	-1.151	1

The parameters are on a log-scale. σ^- and σ^+ indicate the 95% point-wise confidence interval on a log-scale obtained by exploiting the profile likelihood. Parameters with the value $log10(\theta_{opt,i}) = -8.000$ reached the lower bound of the fitting range and are supposed to be zero. Parameters with an infinite confidence interval are not identifiable.

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